THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re application of: Troup, et al.

Confirmation No.: 1877

Application No.: 10/662,678

Group Art Unit: 1654

Filing Date: September 15, 2003

Examiner: Julie Ha

For: NUTRITIONAL COMPOSITIONS

Attorney Docket No.: 9493-US-NP

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPELLANT'S APPEAL BRIEF

Sir:

Appellant submits this Appeal Brief in support of the Notice of Appeal filed February 23, 2011. This Appeal is taken from the Final Rejection dated November 24, 2010 and Advisory Action dated February 17, 2011.

I. REAL PARTY IN INTEREST

The real party in interest for the above-identified patent application on Appeal is Nestec S.A. by virtue of an Assignment dated November 25, 2008 and recorded at reel 021908, frame 0681 in the United States Patent and Trademark Office.

II. RELATED APPEALS AND INTERFERENCES

Appellant's legal representative and the Assignee of the above-identified patent application do not know of any prior or pending appeals, interferences or judicial proceedings which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision with respect to the above-identified Appeal.

III. STATUS OF CLAIMS

Claims 1-4, 6-14 and 16-28 are pending in the above-identified patent application. Claims 6, 12 and 18-22 were previously withdrawn from consideration. Claims 5, 15 and 29 were previously canceled without prejudice or disclaimer. Claims 1-4, 7-11, 13-14, 16-17 and 23-28 stand rejected. Therefore, Claims 1-4, 7-11, 13-14, 16-17 and 23-28 are being appealed in this Brief. A copy of the appealed claims is included in the Claims Appendix.

IV. STATUS OF AMENDMENTS

The Examiner mailed a non-final Office Action on July 20, 2010, in which the Examiner rejected Claims 1-4, 7-11, 13-14, 16-17 and 23-28 under 35 U.S.C. §103; and Claims 1 and 23-25 under 35 U.S.C. §102. Appellant responded to the non-final Office Action on September 13, 2010 in which Appellant amended the claims and argued against the obviousness and anticipation rejections. The Examiner mailed a final Office Action on November 24, 2010, in which the Examiner withdrew the anticipation rejection, amended the previous obviousness rejections and added a new obviousness rejection. On January 10, 2011, Appellant filed a Response to the final Office Action in which Appellant addressed the obviousness rejections. The Examiner mailed a Advisory Action on February 17, 2011. Appellant filed a Notice of Appeal on February 23, 2011. A copy of the non-final Office Action, final Office Action and Advisory Action are attached hereto as Exhibits A, B and C, respectively.

V. SUMMARY OF CLAIMED SUBJECT MATTER

A summary of the invention by way of reference to the drawings and specification for each of the independent claims is provided below.

Independent Claim 1 is directed to a composition comprising: leucine (page 2, lines 23-30 and page 4, lines 1-19), valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids (page 5, lines 1-4), and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof (page 2, lines 23-30 and page 3, lines 17-22) in free and/or salt form (page 2, lines 8-12 and page 3 lines 17-22), wherein said leucine, in free and/or salt form (page 2, lines 23-30 and page 3, lines 7-22), is present in an amount of at least 30% to about 95% by weight based on the weight of total amino acids (page 2, lines 23-34) and wherein said composition provides a ratio of total essential amino acids to total amino acids ranging from about 0.60 to about 0.90 (page 3, lines 29-33 and page 6, lines 20-27).

Independent Claim 2 is directed to a composition comprising: leucine (page 2, lines 23-30 and page 4 lines 1-19), valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids (page 5, lines 1-4), and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof (page 2, lines 23-30 and page 3, lines 17-22) in free and/or salt form (page 2, lines 8-12 and page 3 lines 17-22), wherein total leucine is present in an amount of at least 30% to about 35% by weight based on the weight of total amino acids (page 2, lines 23-30 and page 4 lines 1-19) and wherein said composition provides a ratio of total essential amino acids to total amino acids ranging from about 0.60 to about 0.90 (page 3, lines 29-33 and page 6, lines 20-27).

Independent Claim 3 is directed to a composition comprising:

a) leucine (page 2, lines 23-30 and page 4 lines 1-19), valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids (page 5, lines 1-4), and at least one essential amino acid in free form and/or salt form selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof (page 2, lines 23-30 and page 3, lines 17-22) and, optionally, at least one conditionally essential amino acid selected from the group consisting of tyrosine, cysteine,

arginine, glutamine, and combinations thereof in free form and/or salt form (page 3, lines 17-22 and page 5 lines 6-8), and

b) at least one intact protein selected from the group consisting of casein, whey protein, soy protein, collagen, wheat protein, and combinations thereof (page 3, lines 2-5 and page 6, lines 8-18), wherein said composition provides a ratio of total essential amino acids (page 3, lines 29-33 and page 6, lines 20-27) and, optionally, conditionally essential amino acids to total amino acids ranging from about 0.60 to about 0.90 (page 3, lines 29-33 and page 6, lines 20-27) and wherein said leucine, in free and/or salt form (page 2, lines 23-30 and page 3, lines 7-22), is present in an amount of at least 30% by weight based on the weight of intact protein (page 6, lines 8-18), wherein the ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3 (page 4, lines 5-15).

Independent Claim 17 is directed to a kit comprising:

a) a first composition comprising:

1) leucine (page 2, lines 23-30 and page 4 lines 1-19), valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids (page 5, lines 1-4), and at least one essential amino acid in free form and/or salt form selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof (page 2, lines 23-30 and page 3, lines 17-22) and, optionally, at least one conditionally essential amino acid selected from the group consisting of tyrosine, cysteine, arginine and glutamine in free form and/or salt form (page 3, lines 17-22 and page 5 lines 6-8), and

2) at least one intact protein selected from the group consisting of casein, whey protein, soy protein, collagen, wheat protein, and combinations thereof (page 3, lines 2-5 and page 6, lines 8-18), wherein said composition provides a ratio of total essential amino acids (page 3, lines 29-33 and page 6, lines 20-27) and, optionally, conditionally essential amino acids to total amino acids ranging from about 0.60 to about 0.90 (page 3, lines 29-33 and page 6, lines 20-27) and wherein said leucine, in free and/or salt form(page 2, lines 23-30 and page 3, lines 7-22), is present in an amount of at least 30% by weight based on the weight of intact protein (page 6, lines 8-18), wherein the ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3 (page 4, lines 5-15); and

b) a second composition comprising an anti-cancer drug (page 14, lines 24-33), wherein said anticancer drug is selected from the group consisting of 5-fluorouracil, mitomycin-C, adriamycin, chloroethyl nitrosureas, methotrexate, and combinations thereof (page 14, lines 24-33).

Independent Claim 23 is directed to a composition consisting essentially of: leucine (page 2, lines 23-30 and page 4 lines 1-19), valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids (page 5, lines 1-4), and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof (page 2, lines 23-30 and page 3, lines 17-22) in free and/or salt form (page 2, lines 8-12 and page 3 lines 17-22), wherein leucine, in free and/or salt form (page 2, lines 23-30 and page 3, lines 7-22), is present in an amount of at least 30% by weight based on the weight of total amino acids (page 2, lines 23-30 and page 4 lines 1-19).

Independent Claim 24 is directed to a composition consisting essentially of: leucine (page 2, lines 23-30 and page 4 lines 1-19), valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids (page 5, lines 1-4), and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof (page 2, lines 23-30 and page 3, lines 17-22) in free and/or salt form (page 2, lines 8-12 and page 3 lines 17-22), wherein total leucine is present in an amount of at least 30% by weight based on the weight of total amino acids (page 2, lines 23-30 and page 4 lines 1-19).

Independent Claim 25 is directed to a composition consisting essentially of:

a) leucine (page 2, lines 23-30 and page 4 lines 1-19), valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids (page 5, lines 1-4), and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof (page 2, lines 23-30 and page 3, lines 17-22) and, optionally, at least one conditionally essential amino acid selected from the group consisting of tyrosine, cysteine, arginine, glutamine, and combinations thereof in free form and/or salt form (page 3, lines 17-22 and page 5 lines 6-8), wherein said leucine, in free and/or salt form (page 2, lines 23-30 and page 3, lines 7-22), is present in an amount of at least 30% (page 2, lines 23-30 and page 4 lines 1-19), and

b) at least one intact protein selected from the group consisting of casein, whey protein, soy protein, collagen, wheat protein, and combinations thereof (page 3, lines 2-5 and page 6, lines 8-18), wherein said composition provides a ratio of total essential amino acids (page 3, lines 29-33 and page 6, lines 20-27) and, optionally, conditionally essential amino acids (page 3, lines 29-33 and page 6, lines 20-27), to total amino acids ranging from about 0.60 to about 0.90 (page 3, lines 29-33 and page 6, lines 20-27), wherein the ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3 (page 4, lines 5-15).

Independent Claim 28 is directed to a kit comprising:

a) a first composition consisting essentially of:

1) leucine (page 2, lines 23-30 and page 4 lines 1-19), valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids (page 5, lines 1-4), and at least one essential amino acid in free form and/or salt form selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof (page 2, lines 23-30 and page 3, lines 17-22), and, optionally, at least one conditionally essential amino acid selected from the group consisting of tyrosine, cysteine, arginine, glutamine, and combinations thereof, in free form and/or salt form (page 3, lines 17-22 and page 5 lines 6-8), wherein said leucine, in free and/or salt form (page 2, lines 23-30 and page 3, lines 7-22), is present in an amount of at least 30% (page 2, lines 23-30 and page 4 lines 1-19), and

2) at least one intact protein selected from the group consisting of casein, whey protein, soy protein, collagen, wheat protein, and combinations thereof (page 3, lines 2-5 and page 6, lines 8-18), wherein said composition provides a ratio of total essential amino acids and, optionally, conditionally essential amino acids to total amino acids ranging from about 0.60 to about 0.90 (page 3, lines 29-33 and page 6, lines 20-27), wherein the ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3 (page 4, lines 5-15); and

b) a second composition comprising an anti-cancer drug (page 14, lines 24-33), wherein said anticancer drug is selected from the group consisting of 5-fluorouracil, mitomycin-C, adriamycin, chloroethyl nitrosureas, methotrexate, and combinations thereof (page 14, lines 24-33).

Although specification citations are given in accordance with C.F.R. 1.192(c), these reference numerals and citations are merely examples of where support may be found in the specification for the terms used in this section of the Brief. There is no intention to suggest in any way that the terms of the claims are limited to the examples in the specification. As demonstrated by the references numerals and citations, the claims are fully supported by the specification as required by law. However, it is improper under the law to read limitations from the specification into the claims. Pointing out specification support for the claim terminology as is done here to comply with rule 1.192(c) does not in any way limit the scope of the claims to those examples from which they find support. Nor does this exercise provide a mechanism for circumventing the law precluding reading limitations into the claims from the specification. In short, the references numerals and specification citations are not to be construed as claim limitations or in any way used to limit the scope of the claims.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Claims 1-4, 7-11, 13-14, 16-17 and 23-28 are rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 6,077,828 to Abbruzzese, et al. ("*Abbruzzese*") as evidenced by U.S. Patent No. 4,112,123 Roberts ("*Roberts*") in view of U.S. Patent No. 6,420,342 to Hageman et al. ("*Hageman*"), U.S. Patent No. 6,953,679 to Salvati, et al. ("*Salvati*"), and U.S. Patent No. 6,203,820 to Vickery ("*Vickery*"). Copies of *Abbruzzese*, *Roberts*, *Hageman*, *Salvati* and *Vickery* are attached hereto as Exhibits D, E, F, G and H in the Evidence Appendix.

2. Claims 1-4, 7-11, 13-14, 16-17 and 23-26 are rejected under 35 U.S.C. §103(a) as being unpatentable over *Abbruzzese* as evidence by *Roberts* in view of U.S. Publication No. 2003/0119888 to Allen et al. ("*Allen*") and Sports Supplement Review, 1997, pp. 66-70 to Phillips Bill ("*Phillips*") and *Vickery*. Copies of *Allen* and *Phillips* are attached hereto as Exhibits I and J in the Evidence Appendix.

3. Claims 1 and 23-25 are rejected under 35 U.S.C. 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 4,544,568 to Heyland et al. ("*Heyland*") in view of *Vickery*. A copy of *Heyland* is attached hereto as Exhibit K in the Evidence Appendix.

VII. ARGUMENT

A. LEGAL STANDARDS

Obviousness under 35 U.S.C. § 103

The Federal Circuit has held that the legal determination of an obviousness rejection under 35 U.S.C. § 103 is:

> whether the claimed invention as a whole would have been obvious to a person of ordinary skill in the art at the time the invention was made...The foundational facts for the prima facie case of obviousness are: (1) the scope and content of the prior art; (2) the difference between the prior art and the claimed invention; and (3) the level of ordinary skill in the art...Moreover, objective indicia such as commercial success and long felt need are relevant to the determination of obviousness...Thus, each obviousness determination rests on its own facts.

In re Mayne, 41 U.S.P.Q. 2d 1451, 1453 (Fed. Cir. 1997).

In making this determination, the Patent Office has the initial burden of proving a *prima facie* case of obviousness. *In re Rijckaert*, 28 U.S.P.Q. 2d 1955, 1956 (Fed. Cir. 1993). This burden may only be overcome "by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings." *In re Fine*, 5 U.S.P.Q. 2d 1596, 1598 (Fed. Cir. 1988). "If the examination at the initial stage does not produce a prima facie case of unpatentability, then without more the applicant is entitled to grant of the patent." *In re Oetiker*, 24 U.S.P.Q. 2d 1443, 1444 (Fed. Cir. 1992).

Moreover, the Patent Office must provide explicit reasons why the claimed invention is obvious in view of the prior art. The Supreme Court has emphasized that when formulating a rejection under 35 U.S.C. § 103(a) based upon a combination of prior art elements it remains necessary to identify the reason why a person of ordinary skill in the art would have combined the prior art elements in the manner claimed. *KSR v. Teleflex*, 127 S. Ct. 1727 (2007).

Of course, references must be considered as a whole and those portions teaching against or away from the claimed invention must be considered. *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve Inc.*, 796 F.2d 443 (Fed. Cir. 1986). "A prior art reference may be considered

to teach away when a person of ordinary skill, upon reading the reference would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the Applicant." *Monarch Knitting Machinery Corp. v. Fukuhara Industrial Trading Co., Ltd.*, 139 F.3d 1009 (Fed. Cir. 1998), quoting, *In re Gurley*, 27 F.3d 551 (Fed. Cir. 1994).

B. THE CLAIMED INVENTION

Independent Claim 1 is directed to a composition including leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof in free and/or salt form. The leucine, in free and/or salt form, is present in an amount of at least 30% to about 95% by weight based on the weight of total amino acids. The composition also provides a ratio of total essential amino acids to total amino acids ranging from about 0.60 to about 0.90.

Independent Claim 2 is directed to a composition including leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof in free and/or salt form. Total leucine is present in an amount of at least 30% to about 35% by weight based on the weight of total amino acids. The composition provides a ratio of total essential amino acids to total amino acids ranging from about 0.60 to about 0.90.

Independent Claim 3 is directed to a composition having a) leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid in free form and/or salt form selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof. The composition further includes, optionally, at least one conditionally essential amino acid selected from the group consisting of tyrosine, cysteine, arginine, glutamine, and combinations thereof in free form and/or salt form. The composition further includes b) at least one intact protein selected from the group consisting of casein, whey protein, soy protein, collagen, wheat protein, and combinations thereof. The composition provides a ratio of total

essential amino acids and, optionally, conditionally essential amino acids to total amino acids ranging from about 0.60 to about 0.90. The leucine, in free and/or salt form, is present in an amount of at least 30% by weight based on the weight of intact protein, wherein the ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3.

Independent Claim 17 is directed to a kit including a) a first composition having 1) leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid in free form and/or salt form selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof. The first composition further includes, optionally, at least one conditionally essential amino acid selected from the group consisting of tyrosine, cysteine, arginine and glutamine in free form and/or salt form. The first composition also includes 2) at least one intact protein selected from the group consisting of casein, whey protein, soy protein, collagen, wheat protein, and combinations thereof. The composition provides a ratio of total essential amino acids and, optionally, conditionally essential amino acids to total amino acids ranging from about 0.60 to about 0.90. The leucine, in free and/or salt form, is present in an amount of at least 30% by weight based on the weight of intact protein, wherein the ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3. The kit further includes b) a second composition comprising an anti-cancer drug. The anticancer drug is selected from the group consisting of 5-fluorouracil, mitomycin-C, adriamycin, chloroethyl nitrosureas, methotrexate, and combinations thereof.

Independent Claim 23 is directed to a composition consisting essentially of leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof in free and/or salt form. The leucine, in free and/or salt form, is present in an amount of at least 30% by weight based on the weight of total amino acids.

Independent Claim 24 is directed to a composition consisting essentially of leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof in

free and/or salt form. Total leucine is present in an amount of at least 30% by weight based on the weight of total amino acids.

Independent Claim 25 is directed to a composition consisting essentially of a) leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof. The composition further includes, optionally, at least one conditionally essential amino acid selected from the group consisting of tyrosine, cysteine, arginine, glutamine, and combinations thereof in free form and/or salt form. The leucine, in free and/or salt form, is present in an amount of at least 30%,. The composition further includes b) at least one intact protein selected from the group consisting of casein, whey protein, soy protein, collagen, wheat protein, and combinations thereof. The composition provides a ratio of total essential amino acids and, optionally, conditionally essential amino acids, to total amino acids ranging from about 0.60 to about 0.90. The ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3.

Independent Claim 28 is directed to a kit including a) a first composition consisting essentially of 1) leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid in free form and/or salt form selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof. The first composition further includes, optionally, at least one conditionally essential amino acid selected from the group consisting of tyrosine, cysteine, arginine, glutamine, and combinations thereof, in free form and/or salt form. The leucine, in free and/or salt form, is present in an amount of at least 30%. The first composition further includes 2) at least one intact protein selected from the group consisting of casein, whey protein, soy protein, collagen, wheat protein, and combinations thereof. The first composition provides a ratio of total essential amino acids and, optionally, conditionally essential amino acids to total amino acids ranging from about 0.60 to about 0.90. The ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3. The kit further includes b) a second composition including an anti-cancer drug. The anticancer drug is selected from the group consisting of 5-fluorouracil, mitomycin-C, adriamycin, chloroethyl nitrosureas, methotrexate, and combinations thereof.

C. THE REJECTION OF CLAIMS 1-4, 7-11, 13-14, 16-17 AND 23-28 UNDER 35 U.S.C. §103(a) SHOULD BE REVERSED BECAUSE THE EXAMINER HAS FAILED TO ESTABLISH A *PRIMA FACIE* CASE OF OBVIOUSNESS

Appellant respectfully submits that the obviousness rejection of Claims 1-4, 7-11, 13-14, 16-17 and 23-28 should be reversed because the Examiner has failed to establish a *prima facie* case of obviousness. In the final Office Action, the Examiner asserts that the combination of *Abbruzzese*, *Roberts*, *Hageman*, *Salvati* and *Vickery* renders the claimed subject matter obvious. See, final Office Action, pages 3-13. However, the Examiner has failed to establish a *prima facie* case of obviousness because the cited references fail to disclose each and every element of the present claims.

1. The Presently Claimed Compositions and Advantages of Same

Independent Claims 1-3, 17, 23-25 and 28 recite, in part, compositions having leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof in free and/or salt form, wherein said leucine, in free and/or salt form. Appellant has found that when dietary intake is limited below the optimal level for physiological or patho-physiological reasons, a dietary supplement must be more effective than normal food intake in order to provide a benefit. This is because in this circumstance, when a dietary supplement is given, normal food intake is likely to be reduced by a calorically equivalent amount. Consequently, a supplement designed to limit cancer cachexia, for example, should stimulate muscle protein synthesis to a greater extent than normal food intake and should not interfere with the response to meal intake. Trials of conventional nutritional supplements in patients with cancer cachexia have failed to show appreciable benefit in terms of weight gain or quality of life. Accordingly, there is a need for effective nutritional approaches capable of treating, preventing or ameliorating the effects of tumor-induced weight loss due to, for example, cancer cachexia and/or anorexia.

Appellant has surprisingly found that a formulation containing free essential amino acids as compared to a formulation containing free essential and non-essential amino acids or intact protein alone is optimal. See, specification, Examples 1-2. Appellant has also found that nutritional compositions comprising a mixture of essential amino acids in free form and/or in salt form that has particularly high amounts of leucine had a stimulatory effect on muscle protein synthesis. See, specification, Example 3.

In addition, Appellant has surprisingly and unexpectedly found that particularly useful compositions for promotion of muscle protein synthesis or controlling tumor-induced weight loss, such as cachexia (e.g. cancer cachexia) may be obtained by combining essential amino acids in free form and/or in salt form with intact protein. See, specification, Example 2. The effect of such a combination is greater than the effect that can be achieved with either type of combination partner alone. In contrast, Appellant respectfully submits that the cited references fail to disclose or suggest every element of the present claims.

2. The Cited References Fail to Disclose or Suggest Each and Every Element of the Present Claims

Abbruzzese, *Roberts*, *Hageman*, *Salvati* and *Vickery* all fail to disclose or suggest compositions having leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from the histidine, and combinations thereof in free and/or salt form, wherein said leucine, in free and/or salt form as required, in part, by independent Claims 1-3, 17, 23-25 and 28. Instead, *Abbruzzese* is directed to methods and nutritional compositions for preventing and treating cachexia and anorexia. The compositions of *Abbruzzese* include effective amounts of (1) $\omega 3$ fatty acids, such as α-linolenic acid, stearidonic acid, eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid or mixtures thereof; (2) branched-chain amino acids, such as valine, leucine, isoleucine or mixtures thereof; with or without reduced levels of tryptophan and 5-hydroxytryptophan; and (3) an anti-oxidant system selected from the group consisting of beta-carotene, vitamin C, vitamin E, selenium, or mixtures thereof. See, e.g., *Abbruzzese*, column 3, lines 15-56. However, at no place in the disclosure does *Abbruzzese* disclose or suggest

compositions containing about 8% to about 10% of valine as required, in part, by the present claims. Indeed, at best, *Abbruzzese* discloses only 5.9% valine. See, *Abbruzzese*, Table 4.

Hageman generally describes a nutritional, pharmaceutical or dietetic preparation that includes effective amounts of ribose and folic acid, optionally combined with other components, such as niacin, histidine, glutamine, orotate, vitamin B6 and other components. See, *Hageman*, column 5, lines 8-52. *Hageman* also discloses products having the following mixture of amino acids as beneficial for muscle growth when consumed in an amount of more than 2 and preferably more than 4 g per daily dose: 3-10 wt % histidine, 5-15 wt % isoleucine, 10-23 wt % leucine, 10-23 wt % lysine, 5-15 wt % methionine, 5-15 wt % phenylalanine, 5-15 wt % threonine. See, *Hageman*, column 6, line 62-column 7, line 1. At no place in the disclosure does *Hageman* disclose or suggest compositions containing about 8% to about 10% of valine as required, in part, by the present claims. Indeed, at best, *Hageman* discloses only 3.5% valine. See, *Hageman*, Example 2.

Salvati generally describes fused cyclic compounds and methods of using such compounds in the treatment of nuclear hormone receptor-associated diseases such as cancer and immune disorders and pharmaceutical compositions containing such compounds. See, *Salvati*, Abstract. At no place in the disclosure does *Salvati* disclose or suggest compositions containing about 8% to about 10% of valine as required, in part, by the present claims.

Roberts is entirely directed to a balanced food composition for oral ingestion and producing low residues and diminished stoolings. See, *Roberts*, Abstract. *Roberts* is cited by the Examiner for the disclosure of the amounts of amino acids in whey proteins. At no place in the disclosure does *Roberts* disclose or suggest compositions containing about 8% to about 10% of valine as required, in part, by the present claims.

Vickery is entirely directed toward compositions for enhancing protein anabolism and detoxification comprising molybdenum and at least two amino acids. See, *Vickery*, Abstract. Although *Vickery* discloses valine as a potential amino acid, at no place in the disclosure does *Vickery* disclose or suggest compositions containing 8% to about 10% of valine as required, in part, by the present claims.

The Examiner asserts that "*Vickery* teaches that L-valine aids in wound healing, muscle growth and liver diseases. L-valine is present in the composition in an amount of from about 7%

to about 10% by weight, from about 8% to about 9% by weight." See, final Office Action, page 23, lines 16-18. Appellant respectfully submits that *Vickery* still fails to disclose or suggest the presently claimed amounts of valine. For example, the present claims expressly require that the valine is present in an amount from about 8% to about 10% <u>by weight of total amino acids</u>.

In contrast, *Vickery* expressly discloses that valine may be present in amounts of about 7% to about 10% "based on the total weight of the composition." See, *Vickery*, column 2, lines 8-25. *Vickery* also expressly states that L-Valine may be present in amounts from 7% to about 10%, more preferably about 8% to about 9% "based on the total weight of the active ingredients in the composition." *Vickery* further defines the "active ingredients" as including various amino acids, molybdenum, creatinine, creatine, monohydrate, sulfur, methylsulfonylmethane, powdered egg white or powdered milk, and powdered enzymes. See, *Vickery*, column 5, lines 58-67. As such, it is clear that the "active ingredients" of *Vickery* include minerals, powdered dairy components and enzymes, among other ingredients. Thus, the amounts of valine disclosed in *Vickery* are not based on the weight of total amino acids, as is required, in part, by the present claims.

For at least these reasons, Appellant respectfully submits that the obviousness rejections are improper and that the cited references fail to disclose or suggest each and every element of the present claim.

Accordingly, Appellant respectfully requests that the obviousness rejections of Claims 1-4, 7-11, 13-14, 16-17 and 23-28 under 35 U.S.C. §103 be reconsidered and withdrawn.

D. <u>THE REJECTION OF CLAIMS 1-4, 7-11, 13-14, 16 AND 23-26 UNDER 35 U.S.C. §103(a) SHOULD BE REVERSED BECAUSE THE EXAMINER HAS FAILED TO ESTABLISH A *PRIMA FACIE* CASE OF OBVIOUSNESS</u>

Appellant respectfully submits that the obviousness rejection of Claims 1-4, 7-11, 13-14, 16 and 23-26 should be reversed because the Examiner has failed to establish a *prima facie* case of obviousness. In the final Office Action, the Examiner asserts that the combination of *Abbruzzese*, *Roberts*, *Allen*, *Phillips* and *Vickery* renders the claimed subject matter obvious. See, final Office Action, pages 13-27. However, the Examiner has failed to establish a *prima*

facie case of obviousness because the cited references fail to disclose each and every element of the present claims.

1. The Presently Claimed Compositions and Advantages of Same

Independent Claims 1-3 and 23-25 recite, in part, compositions having leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof in free and/or salt form, wherein said leucine, in free and/or salt form. Appellant has found that when dietary intake is limited below the optimal level for physiological or patho-physiological reasons, a dietary supplement must be more effective than normal food intake in order to provide a benefit. This is because in this circumstance, when a dietary supplement is given, normal food intake is likely to be reduced by a calorically equivalent amount. Consequently, a supplement designed to limit cancer cachexia, for example, should stimulate muscle protein synthesis to a greater extent than normal food intake and should not interfere with the response to meal intake. Trials of conventional nutritional supplements in patients with cancer cachexia have failed to show appreciable benefit in terms of weight gain or quality of life. Accordingly, there is a need for effective nutritional approaches capable of treating, preventing or ameliorating the effects of tumor-induced weight loss due to, for example, cancer cachexia and/or anorexia.

Appellant has surprisingly found that a formulation containing free essential amino acids as compared to a formulation containing free essential and non-essential amino acids or intact protein alone is optimal. See, specification, Examples 1-2. Appellant has also found that nutritional compositions comprising a mixture of essential amino acids in free form and/or in salt form that has particularly high amounts of leucine had a stimulatory effect on muscle protein synthesis. See, specification, Example 3.

In addition, Appellant has surprisingly and unexpectedly found that particularly useful compositions for promotion of muscle protein synthesis or controlling tumor-induced weight loss, such as cachexia (e.g. cancer cachexia) may be obtained by combining essential amino acids in free form and/or in salt form with intact protein. See, specification, Example 2. The effect of such a combination is greater than the effect that can be achieved with either type of

combination partner alone. In contrast, Appellant respectfully submits that the cited references fail to disclose or suggest every element of the present claims.

2. <u>The Cited References Fail to Disclose or Suggest Each and Every Element of the Present Claims</u>

Abbruzzese, *Roberts*, *Allen*, *Phillips* and *Vickery* all fail to disclose or suggest compositions having leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from the histidine, and combinations thereof in free and/or salt form, wherein said leucine, in free and/or salt form as required, in part, by independent Claims 1-3, 17, 23-25 and 28. Instead, *Abbruzzese* is directed to methods and nutritional compositions for preventing and treating cachexia and anorexia. The compositions of *Abbruzzese* include effective amounts of (1) ω3 fatty acids, such as α-linolenic acid, stearidonic acid, eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid or mixtures thereof; (2) branched-chain amino acids, such as valine, leucine, isoleucine or mixtures thereof; with or without reduced levels of tryptophan and 5-hydroxytryptophan; and (3) an anti-oxidant system selected from the group consisting of beta-carotene, vitamin C, vitamin E, selenium, or mixtures thereof. See, e.g., *Abbruzzese*, column 3, lines 15-56. However, at no place in the disclosure does *Abbruzzese* disclose or suggest compositions containing about 8% to about 10% of valine as required, in part, by the present claims. Indeed, at best, *Abbruzzese* discloses only 5.9% valine. See, *Abbruzzese*, Table 4.

Roberts is entirely directed to a balanced food composition for oral ingestion and producing low residues and diminished stoolings. See, *Roberts*, Abstract. *Roberts* is cited by the Examiner for the disclosure of the amounts of amino acids in whey proteins. At no place in the disclosure does *Roberts* disclose or suggest compositions containing about 8% to about 10% of valine as required, in part, by the present claims.

Allen is entirely directed to a composition for stimulating muscle growth having an effective amount of L-arginine. See, *Allen*, Abstract. As shown by Example 1 of *Allen*, a preferred composition of the invention included about 0.57% leucine. At no place in the disclosure does *Allen* disclose or suggest compositions containing about 8% to about 10% of valine as required, in part, by the present claims.

Phillips is cited solely for the teaching that beta-hydroxy beta-methylbutyrate (HMB) is a metabolite of leucine and may help to build muscle. See, Office Action, page 36, lines 4-8. At no place in the disclosure does *Phillips* disclose or suggest compositions containing about 8% to about 10% of valine as required, in part, by the present claims.

Vickery is entirely directed toward compositions for enhancing protein anabolism and detoxification comprising molybdenum and at least two amino acids. See, *Vickery*, Abstract. Although *Vickery* discloses valine as a potential amino acid, at no place in the disclosure does *Vickery* disclose or suggest compositions containing 8% to about 10% of valine as required, in part, by the present claims.

The Examiner asserts that "*Vickery* teaches that L-valine aids in wound healing, muscle growth and liver diseases. L-valine is present in the composition in an amount of from about 7% to about 10% by weight, from about 8% to about 9% by weight." See, final Office Action, page 23, lines 16-18. Appellant respectfully submits that *Vickery* still fails to disclose or suggest the presently claimed amounts of valine. For example, the present claims expressly require that the valine is present in an amount from about 8% to about 10% <u>by weight of total amino acids</u>.

In contrast, *Vickery* expressly discloses that valine may be present in amounts of about 7% to about 10% "based on the total weight of the composition." See, *Vickery*, column 2, lines 8-25. *Vickery* also expressly states that L-Valine may be present in amounts from 7% to about 10%, more preferably about 8% to about 9% "based on the total weight of the active ingredients in the composition." *Vickery* further defines the "active ingredients" as including various amino acids, molybdenum, creatinine, creatine, monohydrate, sulfur, methylsulfonylmethane, powdered egg white or powdered milk, and powdered enzymes. See, *Vickery*, column 5, lines 58-67. As such, it is clear that the "active ingredients" of *Vickery* include minerals, powdered dairy components and enzymes, among other ingredients. Thus, the amounts of valine disclosed in *Vickery* are not based on the weight of total amino acids, as is required, in part, by the present claims.

For at least these reasons, Appellant respectfully submits that the obviousness rejection of Claims 1-4, 7-11, 13-14, 16 and 23-26 is improper and that the cited references fail to disclose or suggest each and every element of the present claims.

Accordingly, Appellant respectfully requests that the obviousness rejections of Claims 1-4, 7-11, 13-14, 16 and 23-26 under 35 U.S.C. §103 be reconsidered and withdrawn.

E. <u>THE REJECTION OF CLAIMS 1 AND 23-25 UNDER 35 U.S.C. §103(a) SHOULD BE REVERSED BECAUSE THE EXAMINER HAS FAILED TO ESTABLISH A *PRIMA FACIE* CASE OF OBVIOUSNESS</u>

Appellant respectfully submits that the obviousness rejection of Claims 1 and 23-25 should be reversed because the Examiner has failed to establish a *prima facie* case of obviousness. In the final Office Action, the Examiner asserts that the combination of *Heyland* and *Vickery* renders the claimed subject matter obvious. See, final Office Action, pages 27-30. However, the Examiner has failed to establish a *prima facie* case of obviousness because the cited references fail to disclose each and every element of the present claims.

1. <u>The Presently Claimed Compositions and Advantages of Same</u>

Independent Claims 1 and 25 recite, in part, compositions having leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, a ratio of total essential amino acids and, optionally, conditionally essential amino acids to total amino acids ranging from about 0.60 to about 0.90 and wherein the ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3. Independent Claims 23-24 recite, in part, compositions <u>consisting essentially of</u> leucine in free and/or salt form, is present in an amount of at least 30% by weight based on the weight of total amino acids, and valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids. As discussed above, Appellant has surprisingly found that a formulation containing free essential amino acids as compared to a formulation containing free essential and non-essential amino acids or intact protein alone is optimal. See, specification, Examples 1-2. Appellant has also found that nutritional compositions comprising a mixture of essential amino acids in free form and/or in salt form that has particularly high amounts of leucine had a stimulatory effect on muscle protein synthesis. See, specification, Example 3. In contrast, Appellant respectfully submits that *Heyland* and *Vickery* fail to disclose or suggest each and every element of the present claims.

2. The Cited References Fail to Disclose or Suggest Each and Every Element of the Present Claims

Heyland and *Vickery* fail to disclose or suggest compositions having leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, a ratio of total essential amino acids and, optionally, conditionally essential amino acids to total amino acids ranging from about 0.60 to about 0.90 and wherein the ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3 as required, in part, by independent Claims 1 and 25. *Heyland* and *Vickery* also fail to disclose or suggest compositions consisting essentially of leucine in free and/or salt form, present in an amount of at least 30% by weight based on the weight of total amino acids, and valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, as required, in part, by independent Claims 23 and 24. Instead, *Heyland* discloses all essential amino and fails to recite any non-essential amino acids at any place in the disclosure. As such, *Heyland* cannot disclose or suggest a ratio of total essential amino acids and, optionally, conditionally essential amino acids to total amino acids ranging from about 0.60 to about 0.90 as required, in part, by independent Claims 1 and 25. Further, the Examiner even admits that *Heyland* "does not teach about 8% to about 10% valine." See, final Office Action, page 27, lines 21-22.

Moreover, as noted by the Examiner, *Heyland* expressly discloses a composition having 150 g of technical leucine, 70 g of monosodium glutamate, 40 g of sodium chloride, 180 g of whey powder and 160 g of a casein hydrolysate. See, *Heyland*, column 6, lines 55-60. In contrast, the present claims use the transitional phrase of "consisting essentially of," which limits the scope of a claim to the specific materials or steps and those that do not materially affect the basic or novel characteristics of the claimed invention. See, MPEP 2111.02; *In re Herz*, 537 F.2d 549, 551-52 (CCPA 1976). The Federal Circuit has also characterized a "consisting essentially of" claim as occupying a middle ground between closed claims of "consisting of" format and fully open claims of "comprising" format. See, *PPG Industries v. Guardian Industries*, 156 F.3d 1351, 1354 (Fed. Cir. 1998).

Therefore, with regard to the present claims, the "consisting essentially of" language limits the composition to containing leucine, valine and an essential amino acid (Claims 1 and 23-24) or leucine, valine, an essential amino acid and an intact protein (Claim 25), and those

materials that do not materially affect the basic or novel characteristics of the claimed invention. Appellant respectfully submits that the additional composition elements disclosed in *Heyland* materially affects the basic or novel characteristics of the claimed compositions. Accordingly, the "consisting essentially of" language of the present claims is closed language that excludes the additional elements of the compositions described in *Heyland.* As such, *Heyland* fails to disclose each and every element of the present claims and fail to even recognize the advantages of the presently claimed subject matter.

Vickery is entirely directed toward compositions for enhancing protein anabolism and detoxification comprising molybdenum and at least two amino acids. See, *Vickery*, Abstract. Although *Vickery* discloses valine as a potential amino acid, at no place in the disclosure does *Vickery* disclose or suggest compositions containing 8% to about 10% of valine as required, in part, by the present claims.

The Examiner asserts that "*Vickery* teaches that L-valine aids in wound healing, muscle growth and liver diseases. L-valine is present in the composition in an amount of from about 7% to about 10% by weight, from about 8% to about 9% by weight." See, final Office Action, page 23, lines 16-18. Appellant respectfully submits that *Vickery* still fails to disclose or suggest the presently claimed amounts of valine. For example, the present claims expressly require that the valine is present in an amount from about 8% to about 10% <u>by weight of total amino acids</u>.

In contrast, *Vickery* expressly discloses that valine may be present in amounts of about 7% to about 10% "based on the total weight of the composition." See, *Vickery*, column 2, lines 8-25. *Vickery* also expressly states that L-Valine may be present in amounts from 7% to about 10%, more preferably about 8% to about 9% "based on the total weight of the active ingredients in the composition." *Vickery* further defines the "active ingredients" as including various amino acids, molybdenum, creatinine, creatine, monohydrate, sulfur, methylsulfonylmethane, powdered egg white or powdered milk, and powdered enzymes. See, *Vickery*, column 5, lines 58-67. As such, it is clear that the "active ingredients" of *Vickery* include minerals, powdered dairy components and enzymes, among other ingredients. Thus, the amounts of valine disclosed in *Vickery* are not based on the weight of total amino acids, as is required, in part, by the present claims.

For at least these reasons, Appellant respectfully submits that the obviousness rejection of Claims 1 and 23-25 is improper and that *Heyland* and *Vickery* fail to render the presently claimed subject matter obvious.

Accordingly, Appellant respectfully requests that the obviousness rejection of Claims 1 and 23-25 under 35 U.S.C. §103 be reconsidered and withdrawn.

VIII. CONCLUSION

Appellant respectfully submits that the Examiner has failed to establish a *prima facie* case of obviousness under 35 U.S.C. §103(a) with respect to the rejections of Claims 1-4, 7-11, 13-14, 16-17 and 23-28. Accordingly, Appellant respectfully submits that the obviousness rejections are erroneous in law and in fact and should, therefore, be reversed by this Board.

The Director is authorized to charge $540.00 for the Appeal Brief and any additional fees which may be required, or to credit any overpayment to Deposit Account No. 02-1818. If such a withdrawal is made, please indicate the Attorney Docket No. 3712036-00711 on the account statement.

Respectfully submitted,

K&L GATES LLP

BY ______________________

Robert M. Barrett
Reg. No. 30,142
Customer No. 29157
Phone No. 312-807-4204

Dated: April 21, 2011

CLAIMS APPENDIX

PENDING CLAIMS ON APPEAL OF U.S. PATENT APPLICATION SERIAL NO. 10/662,678

1. A composition comprising: leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof in free and/or salt form, wherein said leucine, in free and/or salt form, is present in an amount of at least 30% to about 95% by weight based on the weight of total amino acids and wherein said composition provides a ratio of total essential amino acids to total amino acids ranging from about 0.60 to about 0.90

2. A composition comprising: leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof in free and/or salt form, wherein total leucine is present in an amount of at least 30% to about 35% by weight based on the weight of total amino acids and wherein said composition provides a ratio of total essential amino acids to total amino acids ranging from about 0.60 to about 0.90.

3. A composition comprising:

a) leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid in free form and/or salt form selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof and, optionally, at least one conditionally essential amino acid selected from the group consisting of tyrosine, cysteine, arginine, glutamine, and combinations thereof in free form and/or salt form, and

b) at least one intact protein selected from the group consisting of casein, whey protein, soy protein, collagen, wheat protein, and combinations thereof, wherein said composition provides a ratio of total essential amino acids and, optionally, conditionally essential amino acids to total amino acids ranging from about 0.60 to about 0.90 and wherein said leucine, in free and/or salt form, is present in an amount of at least 30% by weight based on the weight of intact protein, wherein the ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3.

4. The composition of claim 3, wherein the essential amino acid comprises leucine and at least one of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, or histidine.

7. The composition of claim 3, further comprising methionine in free and/or salt form in an amount of at least about 0.5% to about 5% by weight based on the weight of total amino acids.

8. The composition of claim 3, further comprising an n-3 polyunsaturated fatty acid.

9. The composition of claim 8, wherein the n-3 polyunsaturated fatty acid is selected from the group consisting of u-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, and combinations thereof.

10. The composition of claim 3, further comprising at least about 1 g of eicosapentaenoic acid per serving or at least about 2 g of eicosapentaenoic acid per dose.

11. The composition of claim 3, further comprising tocopherol.

13. The composition of claim 11, wherein the tocopherol is present in an amount about 50 mg per serving or at least 150 mg per dose.

14. The composition of claim 3, comprising from about 15 g to about 55 g amino acids in free and/or salt form per dose.

16. The composition of claim 3, comprising from about 36 g to about 72 g total essential and/or conditionally essential amino acids per serving.

17. A kit comprising:

a) a first composition comprising:

1) leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid in free form and/or salt form selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof and, optionally, at least one conditionally essential amino acid selected from the group consisting of tyrosine, cysteine, arginine and glutamine in free form and/or salt form, and

2) at least one intact protein selected from the group consisting of casein, whey protein, soy protein, collagen, wheat protein, and combinations thereof, wherein said composition provides a ratio of total essential amino acids and, optionally, conditionally essential amino acids to total amino acids ranging from about 0.60 to about 0.90 and wherein said leucine, in free and/or salt form, is present in an amount of at least 30% by weight based on the weight of intact protein, wherein the ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3; and

b) a second composition comprising an anti-cancer drug, wherein said anticancer drug is selected from the group consisting of 5-fluorouracil, mitomycin-C, adriamycin, chloroethyl nitrosureas, methotrexate, and combinations thereof.

23. A composition consisting essentially of: leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof in free and/or salt form, wherein leucine, in free and/or salt form, is present in an amount of at least 30% by weight based on the weight of total amino acids.

24. A composition consisting essentially of: leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof in free and/or salt form, wherein total leucine is present in an amount of at least 30% by weight based on the weight of total amino acids.

25. A composition consisting essentially of:

a) leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof and, optionally, at least one conditionally essential amino acid selected from the group consisting of tyrosine, cysteine, arginine, glutamine, and combinations thereof in free form and/or salt form, wherein said leucine, in free and/or salt form, is present in an amount of at least 30%, and

b) at least one intact protein selected from the group consisting of casein, whey protein, soy protein, collagen, wheat protein, and combinations thereof, wherein said composition provides a ratio of total essential amino acids and, optionally, conditionally essential amino acids, to total amino acids ranging from about 0.60 to about 0.90, wherein the ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3.

26. The composition of claim 3, comprising from about 12 g to about 15 g leucine in free and/or salt form per dose.

27. The composition of claim 3, further comprising methionine in free and/or salt form in an amount of at least about 5% to about 7% by weight based on the weight of total amino acids.

28. A kit comprising:

a) a first composition consisting essentially of:

1) leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid in free form and/or salt form selected from the group consisting of isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, and combinations thereof, and, optionally, at least one conditionally essential amino acid selected from the group consisting of tyrosine, cysteine, arginine, glutamine, and combinations thereof, in free form and/or salt form, wherein said leucine, in free and/or salt form, is present in an amount of at least 30%, and

2) at least one intact protein selected from the group consisting of casein, whey protein, soy protein, collagen, wheat protein, and combinations thereof, wherein said composition provides a ratio of total essential amino acids and, optionally, conditionally essential amino acids to total amino acids ranging from about 0.60 to about 0.90, wherein the ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3; and

b) a second composition comprising an anti-cancer drug, wherein said anticancer drug is selected from the group consisting of 5-fluorouracil, mitomycin-C, adriamycin, chloroethyl nitrosureas, methotrexate, and combinations thereof.

EVIDENCE APPENDIX

EXHIBIT A: Non-final Office Action dated July 20, 2010

EXHIBIT B: Final Office Action dated November 24, 2010

EXHIBIT C: Advisory Action dated February 17, 2011

EXHIBIT D: U.S. Patent No. 6,077,828 to Abbruzzese, et al. ("*Abbruzzese*")

EXHIBIT E: U.S. Patent No. 4,112,123 Roberts ("*Roberts*")

EXHIBIT F: U.S. Patent No. 6,420,342 to Hageman et al. ("*Hageman*")

EXHIBIT G: U.S. Patent No. 6,953,679 to Salvati, et al. ("*Salvati*")

EXHIBIT H: U.S. Patent No. 6,203,820 to Vickery ("*Vickery*")

EXHIBIT I: U.S. Publication No. 2003/0119888 to Allen et al. ("*Allen*")

EXHIBIT J: Sports Supplement Review, 1997, pp. 66-70 to Phillips Bill ("*Phillips*")

EXHIBIT K: U.S. Patent No. 4,544,568 to Heyland et al. ("*Heyland*")

RELATED PROCEEDINGS APPENDIX

None.

EXHIBIT A

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/662,678	09/15/2003	John P. Troup	8493-US	1877

74476 7590 07/20/2010
Nestle HealthCare Nutrition
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EXAMINER	
HA, JULIE	

ART UNIT	PAPER NUMBER
1654	

NOTIFICATION DATE	DELIVERY MODE
07/20/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdepartment@rd.nestle.com
athena.pretory@rd.nestle.com

Office Action Summary	**Application No.** 10/662,678	**Applicant(s)** TROUP ET AL.	
	Examiner JULIE HA	**Art Unit** 1654	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *26 April 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-4,6-14 and 16-28* is/are pending in the application.

4a) Of the above claim(s) *6,12 and 18-22* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-4,7-11,13,14,16,17 and 23-28* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Amendment after non-final rejection filed on April 26, 2010 is acknowledged. Claims 1-4, 6-14 and 16-28 are pending in this application.

Claims 6, 12 and 18-22 remain withdrawn from further consideration, as being drawn to nonelected inventions and species. Claims 1-4, 7-11, 13-14, 16-17 and 23-28 are examined on the merits in this office action. After further review, a non final office action follows below.

Withdrawn Rejections

1. Rejection of claims 1-4, 7-11, 13-14, 16 and 23-26 under 35 U.S.C. 102(b) as being anticipated by Abbruzzese et al (U.S. Patent No. 6,077,828) is hereby withdrawn in view of Applicant's amendment to the claims.

2. Rejection of claims 1-4, 7-11, 13-14, 16 and 23-26 under 35 U.S.C. 102(a) as being anticipated by Abbruzzese et al (US Patent No. 6,387,883) is hereby withdrawn in view of Applicant’s amendment to the claims.

3. Rejection of claims 1-4, 7-11, 13-14, 16 and 23-26 under 35 U.S.C. 102(e) as being anticipated by Abbruzzese et al (US Patent No. 6,387,883) is hereby withdrawn in view of Applicant’s amendment to the claims.

4. Rejection of claims 1-4, 7-11, 13-14, 16, 23-27 provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-3, 5-11 of copending Application No. 12/110,016, is hereby withdrawn in view of Applicant’s amendment to the claims.

5. Rejection of claims 1-4, 7-11, 13-14, 16, 23-27 provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-2, 5 and 7 of copending Application No. 12/366,520, is hereby withdrawn in view of Applicant's amendment to the claims.

6. Rejection of claims 10, 13-14, 16 and 26 under 35 U.S.C. 112, second paragraph, is hereby withdrawn in view of Applicant's arguments and amendments.

Revised and Maintained Rejection

35 U.S.C. 103

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

For the purpose of this invention, the level of ordinary skill in the art is deemed to be at least that level of skill demonstrated by the patents in the relevant art. Joy Technologies Inc. V. Quigg, 14 USPQ2d 1432 (DC DC 1990). One of ordinary skill in the art is held in accountable not only for specific teachings of references, but also for inferences which those skilled in the art may reasonably be expected to draw. *In re Hoeschele*, 160 USPQ 809, 811 (CCPA 1969). In addition, one of ordinary skill in the art is motivated

by economics to depart from the prior art to reduce costs consistent with desired product properties. _In re Clinton_, 188 USPQ 365, 367 (CCPA 1976);_In re Thompson_, 192 USPQ 275, 277 (CCPA 1976).

8. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

9. (Revised) Claims 1-4, 7-11, 13-14, 16-17 and 23-28 remain rejected under 35 U.S.C. 103(a) as unpatentable over Abbruzzese et al (US Patent No. 6,077,828) as evidenced by Roberts (US Patent No. 4,112,123), in view of Hageman et al (US Patent No. 6,420,342) and Salvati et al (US Patent No. 6,953,679).

10. Abbruzzese et al teach nutritional compositions for the prevention of cachexia and anorexia. The reference teaches a composition comprising effective amounts of ω-3 fatty acids, such as alpha-linolenic acid, stearidonic acid, eicosapentanenoic acid, docosapentaenoic acid, docosahexanoic acid or mixtures thereof; of branched-chain amino acids valine, leucine, isoleucine or mixtures thereof; with or without reduced levels of tryptophan and 5-hydroxytryptophan; and of antioxidant system selected from the group consisting of beta-carotene, vitamin C, vitamin E, selenium or mixtures thereof (see abstract). The reference teaches a liquid nutritional composition comprising (a) at least 1000 mg per liter of ω-3 fatty acids, wherein the weight ratio of ω-6 fatty

acids to ω-3 fatty acids is from about 0.1 to about 1.0; (b) at least 50 grams per liter of a source of amino nitrogen, wherein 15 to 50% by weight of the amino-nitrogen is branched-chain amino acids, and wherein tryptophan is present in an amount less than about 5.0% by weight of the total amino -nitrogen, and (c) at least 1 gram per liter of an antioxidant system comprising beta-carotene, vitamin C, vitamin E and selenium (see claim 1). The reference teaches that the total amount of branched-chain amino acids (“BCAA") useful in the present invention is about 15-50 g/100 g protein (i.e. percent), preferably about 15-25 g/100 g. Thus, an 8 oz container of the nutritional composition would contain up to about 8 g BCAAs per 16 grams of total protein. The daily delivery of BCAAs is about 5-26 g (see column 9, lines 26-31). The reference teaches the branched-chain amino acids valine, leucine, isoleucine or mixtures thereof (see abstract). Therefore, since there is 9.08 g and total of 19.75 g of BCAA, 9.08/19.75 is about 46% of the leucine in the BCAA. Since the reference teaches that the total amount of BCAA useful in the present invention is about 15-50 g/100 g protein, and there is 46% of leucine in the BCAA composition, this implies that there is about 23% of leucine. Additionally, the reference teaches that the nutritional compositions comprises branched-chain amino acids, valine, leucine, isoleucine or mixtures of thereof. Furthermore, the reference teaches that the liquid nutritional composition comprises per liter (a) at least 0.45 gm (450 mg) of ω-3 fatty acids, (b) at least 50 grams of a source of amino-nitrogen wherein 15-50% by weight of the amino-nitrogen is branched-chain amino acids and wherein tryptophan is present in an amount less than about 5.0% of the total amino-nitrogen, and (c) at least 1 gram of an antioxidant (see column 4, lines

20-36). Therefore, if there was 46% of leucine present, and 46% of 50 gram is 23 grams of leucine present in the composition, this implies that there is about 46% of leucine present in the composition.

The reference further teaches that the composition comprises essential amino acids, such as lysine, isoleucine, methionine, phenylalanine, threonine, tryptophan, valine or histidine, and teaches the amino acid profile of a nutritional composition (see Table 4). Since there is at least about 36 g of essential and/or conditionally essential amino acids per serving and about 15-50 g of BCAA per 100 g of protein, meeting the limitation of claims 14 and 16. Furthermore, since the reference teaches that there is at least 50 grams of a source of amino-nitrogen wherein 15-50% by weight of the amino nitrogen is BCAA, and Table 4 indicates that 9.08 gram of leucine is present, according to the calculation above, this equals about 46% of BCAA. Thus, if 50% by weight of the amino nitrogen is BCAA, there is at least 25 grams of BCAA present. And if about 46% of BCAA is leucine, this implies that there is about 11.5 g of leucine present, meeting the limitation of claim 26. The reference teaches 2.78 g of methionine in 100 g of protein, meeting the limitation of at least about 0.5% to about 5% of methionine of claim 7, The reference teaches that the nutritional composition comprises vitamin E (tocopherol (all natural form or d1-alpha-tocopherol acetate) (see Table 6), meeting the limitation of claim 11. The reference teaches that the EPA is in the amount of 1.09 g and DHA is in the amount of 0.46 g (see Table 3), meeting the limitation of claims 8-10. The reference further teaches that for treatment of ulcerative colitis, compositions include a protein source that can be intact or hydrolyzed proteins of high biological value (see

column 3, lines 1-5) and teaches 75% whey protein concentrate as one of the ingredients (see table 7). Furthermore, since the reference teaches a whey protein concentrate, this protein would inherently comprise essential and conditionally essential amino acid profiled, thus meeting the limitation of range from about 0.60 to about 0.90 amino acids.

As evidenced by Roberts (US Patent No. 4,112,123), per 100 g of whey protein, there is 13.0 g of leucine (see Table 1). Since there is at least 9.08 g of leucine to 13.0 g of leucine in 100 g or whey protein, this would meet the limitation of ratio of 1:3 to 3:1. Furthermore, the reference teaches that for example, the daily nutritional management of liver cancer includes administration of 2 to 4 containers of 8 ounces servings (237 mL) of the nutritional composition providing a daily amount of (i) combined EPA and DHA in the range of 3 to 6 g (preferred dosage 3 g), (ii) BCAA in the range of 5 to 25 g (preferred dosage about 10-15 g), (iii) vitamin C in the range of 125 to 500 mg (preferred about 300 mg), (iv) vitamin E (tocopherol) in the range of 50 to 250 IU (preferred 150 IU), (v) beta-carotene in the range of 1250 to 3250 μg (preferred 2500 μg), (vi) selenium in the range of 40 to 60 μg (preferred about 45 μg). The reference teaches that for cancer cachexia and anorexia, the effect of nutritional intervention are monitored at monthly intervals as known in the art, and depending on the results obtained, the therapeutic regimen is developed to maintain and/or boost the weight gain by the patient (see column 15, lines 44-65, Example III). The mass equivalents of 1 IU for vitamin E is 0.667 mg d-alpha-tocopherol, or of 1 mg of d1-alpha-tocopherol acetate

(definition of IU from medicinenet.com). Therefore, 50 to 250 IU would equal to 33.55 mg to 167.75 mg of tocopherol, meeting the limitation of claim 13.

The difference between the reference and the instant claims is that the reference does not teach at least 30% by weight of leucine based on the weight of intact protein, a kit comprising a first composition and a second composition comprising an anti-cancer drug, wherein said anticancer drug is 5-fluorouracil, mitomycin-C, adriamycin, chloroethyl nitrosureas or methotrexate, and that the methionine in free and/or salt from is in an amount of at least about 5% to about 7% by weight on the weight of total amino acids.

11. However, Hageman et al teach a nutritional, pharmaceutical or dietetic preparation can be manufacture in dry form, as bar, as powder, as tablet, and cookie or as cereal (see column 5, lines 60-63). The reference teaches for products for sportsmen the following mixtures of amino acids appeared to be especially beneficial for muscle growth, when consumed in an amount of more than 2 and preferably more than 4 g per daily dose: 3-10 wt% histidine, 5-15% isoleucine, 10-23% % leucine, 10-23% lysine, 5-15% methionine, 5-15 wt % phenylalanine, and 5-15 wt % threonine (see column 6, lines 59-67 and column 7, line 1). Furthermore, the reference teaches that when proteins are included in the nutritional preparations, the amount that is included depends on the application (see column 6, lines 39-41) and the proteins are proteins of dairy, vegetable or animal origin, such as skimmed milk powder, whey powder, egg white powder, potato protein, soy protein, etc., or hydrolysates, or mixtures thereof (see column 6, lines 27-32). The reference teaches that when proteins are included in the

nutritional preparation, the amount that is included depends on the application of the product. In complete formula typically an amount of 5-120 g per daily dose…for young infants the amount will be in the range 5-15 g per daily dose...in complete enteral nutrition for feeding surgery patients, typically 50-120 g per daily dose…In supplement typically 0-60 g protein per daily dose will be included (see column 6, lines 39-50). In regards to claim 25, the claim is drawn to "a composition consisting essentially of..." In regards to claim 27, the claim is drawn to "a kit comprising: a first composition consisting essentially of..." Applicant has not defined what encompasses "consisting essentially of" in the specification. In fact the instant specification does not define the phrase "consisting essentially of". The MPEP states the following: "The transitional phrase ‘consisting essentially of’ limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. A ‘consisting essentially of’ claim occupies the middle ground between closed claims that are written in a consisting of’ format and fully open claims that are drafted in a comprising' format...For the purposes of searching for and applying prior art under 35 U.S.C. 102 and 103, absent a clear indication in the specification or claims of what the basic and novel characteristics actually are, "consisting essentially of” will be construed as equivalent to 'comprising'" (see MPEP 2105). Therefore, claims 25 and 28 have been treated as “a composition comprising…” the same claim language as original claim 3.

12. Furthermore, Salvati et al teach a fused cyclic compound and the use of the fused cyclic compound with a nutritional supplements in combination with whey protein

or casein, amino acids (such as leucine, branched amino acid and hydroxymethylbutyrate), triglycerides, vitamins (e.g., A, B6, B12, folate, C, D, and E), minerals, etc (see column 45, lines 48-56). Furthermore, the reference teaches anti-proliferative agents for use in combination with the compounds such as adriamycin (see column 45, lines 41-43) and anti-cancer agents, such as methotrexate, 5-fluorouracil (see column 46, lines 64-67). The reference teaches a kit comprising a first container (such as a vial) containing a pharmaceutical formulation comprising a compound, a second container (such as a vial) containing a pharmaceutical formulation comprising one or more agents to be used in combination with the compound of the invention (see 47, lines 55-64).

13. Therefore, it would have been obvious for one of ordinary skill in the art to combine the teachings of Abbruzzese et al, Hageman et al and Salvati et al to produce a kit comprising the anti-cancer agent with the nutritional composition, since all of the prior art teach nutritional composition. Salvati et al teach a kit comprising fused cyclic compound, nutritional supplement comprising leucine, whey and protein and any anti-cancer agent and Hageman et al and Abburuzzese et al teach a nutritional composition comprising different amounts of amino acids. One of ordinary skill in the art would be motivated to combine, since Salvati et al teaches such a composition/kit. Furthermore, the composition taught in Abbruzzese, Hageman, and Salvati can be used for the same purpose, for administering nutritional composition for such patients as cancer patients. As evidenced by the instant specification, the compositions of the instant application are for the promotion of muscle protein synthesis and control of tumor-induced weight loss

in patients that are, for example, suffering from cancer cachexia (see abstract). Furthermore, one of ordinary skill in the art would have been motivated to optimize the concentrations of the leucine and methionine, since "it is the normal desire of scientist or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is optimum combination of percentages". The MPEP states: Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. *"[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)* (Claimed process which was performed at a temperature between 40°C and 80°C and an acid concentration between 25% and 70% was held to be prima facie obvious over a reference process which differed from the claims only in that the reference process was performed at a temperature of 100°C and an acid concentration of 10%.); see also Peterson, 315 F.3d at 1330, 65 USPQ2d at 1382 ("*The normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages."); In re Hoeschele, 406 F.2d 1403, 160 USPQ 809 (CCPA 1969)* (Claimed elastomeric polyurethanes which fell within the broad scope of the references were held to be unpatentable thereover because, among other reasons, there was no evidence of the criticality of the claimed ranges of molecular weight or molar proportions.). For more recent cases applying this

principle, see Merck & Co. Inc. v. Biocraft Laboratories Inc., 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), cert. denied, 493 U.S. 975 (1989); *In re Kulling*, 897 F.2d 1147, 14 USPQ2d 1056 (Fed. Cir. 1990); and *In re Geisler*, 116 F.3d 1465, 43 USPQ2d 1362 (Fed. Cir. 1997). Furthermore, Abbruzzese patent '828 teaches that the effect of nutritional intervention on cancer cachexia and anorexia are monitored as known in the art, and depending on the results obtained, the therapeutic regimen is developed to maintain and /or boost weight gain by the patient, with the ultimate goal of achieving tumor regression and complete eradication of cancer cells (see column 15, lines 57-64). Therefore, there is a reasonable expectation of success to optimize the concentrations of the essential amino acid/ leucine/ methionine, since it is "the normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages" and one of ordinary skill in the art would experiment with different concentrations to produce the optimal product. Due to the loss of appetite due to cancer treatment, and due to appetite suppression, nutrition is necessarily a part of treatment process for improving patient's everyday life. For the process of improving and treating cancer and loss of appetite due to cancer, a nutritional requirement would be optimized and adjusted accordingly by those skilled in the art. Abbruzzese teaches that the therapeutic regimen will be developed to maintain and/or boost weight gain, giving motivation to optimize the nutritional content. From the teachings of the references, it is apparent that one of the ordinary skills in the art would have had a reasonable expectation of success in producing the claimed invention. Thus, the

invention as a whole is *prima facie* obvious over the references, especially in the absence of evidence to the contrary. There is a reasonable expectation of success, since Salvati et al teach a kit that can comprise any agent, nutritional supplement for the treatment of cancer (prostate), and Hageman et al and Abbruzzese et al teach a nutritional supplement comprising essential amino acids that is useful in treating variety of diseases, including cancer.

14. (Revised) Claims 1-4, 7-11, 13-14, 16 and 23-26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Abbruzzese et al (US Patent No. 6,077,828, filed with IDS) as evidenced by Roberts (US Patent No. 4,112,123), in view of Allen et al (US 2003/0119888 A1) and Phillips Bill (Sports Supplement Review, 1997, pp. 66-70).

15. Abbruzzese et al teach nutritional compositions for the prevention of cachexia and anorexia. The reference teaches a composition comprising effective amounts of ω-3 fatty acids, such as alpha-linolenic acid, stearidonic acid, eicosapentanenoic acid, docosapentaenoic acid, docosahexanoic acid or mixtures thereof; of branched-chain amino acids valine, leucine, isoleucine or mixtures thereof; with or without reduced levels of tryptophan and 5-hydroxytryptophan; and of antioxidant system selected from the group consisting of beta-carotene, vitamin C, vitamin E, selenium or mixtures thereof (see abstract). The reference teaches a liquid nutritional composition comprising (a) at least 1000 mg per liter of ω-3 fatty acids, wherein the weight ratio of ω-6 fatty acids to ω-3 fatty acids is from about 0.1 to about 1.0; (b) at least 50 grams per liter of a source of amino nitrogen, wherein 15 to 50% by weight of the amino-nitrogen is

branched-chain amino acids, and wherein tryptophan is present in an amount less than about 5.0% by weight of the total amino -nitrogen, and (c) at least 1 gram per liter of an antioxidant system comprising beta-carotene, vitamin C, vitamin E and selenium (see claim 1). The reference teaches that the total amount of branched-chain amino acids (“BCAA") useful in the present invention is about 15-50 g/100 g protein (i.e. percent), preferably about 15-25 g/100 g. Thus, an 8 oz container of the nutritional composition would contain up to about 8 g BCAAs per 16 grams of total protein. The daily delivery of BCAAs is about 5-26 g (see column 9, lines 26-31). The reference teaches the branched-chain amino acids valine, leucine, isoleucine or mixtures thereof (see abstract). Therefore, since there is 9.08 g and total of 19.75 g of BCAA, 9.08/19.75 is about 46% of the leucine in the BCAA. Since the reference teaches that the total amount of BCAA useful in the present invention is about 15-50 g/100 g protein, and there is 46% of leucine in the BCAA composition, this implies that there is at least about 23% of leucine. Additionally, the reference teaches that the nutritional compositions comprises branched-chain amino acids, valine, leucine, isoleucine or mixtures of thereof. As evidenced by Roberts (US Patent No. 4,112,123), per 100 g of whey protein, there is 13.0 g of leucine (see Table 1). Since there is at least 9.08 g of leucine to 13.0 g of leucine in 100 g or whey protein, this would meet the limitation of ratio of 1:3 to 3:1.

Furthermore, the reference teaches that the liquid nutritional composition comprises per liter (a) at least 0.45 gm (450 mg) of ω-3 fatty acids, (b) at least 50 grams of a source of amino-nitrogen wherein 15-50% by weight of the amino-nitrogen is branched-chain amino acids and wherein tryptophan is present in an amount less than

about 5.0% of the total amino-nitrogen, and (c) at least 1 gram of an antioxidant (see column 4, lines 20-36). Therefore, if there was 46% of leucine present, and 46% of 50 gram is 23 grams of leucine present in the composition, this implies that there is about 46% of leucine present in the composition.

The reference further teaches that the composition comprises essential amino acids, such as lysine, isoleucine, methionine, phenylalanine, threonine, tryptophan, valine or histidine, and teaches the amino acid profile of a nutritional composition (see Table 4). Since there is at least about 36 g of essential and/or conditionally essential amino acids per serving and about 15-50 g of BCAA per 100 g of protein, meeting the limitation of claims 14 and 16. Furthermore, since the reference teaches that there is at least 50 grams of a source of amino-nitrogen wherein 15-50% by weight of the amino nitrogen is BCAA, and Table 4 indicates that 9.08 gram of leucine is present, according to the calculation above, this equals about 46% of BCAA. Thus, if 50% by weight of the amino nitrogen is BCAA, there is at least 25 grams of BCAA present. And if about 46% of BCAA is leucine, this implies that there is about 11.5 g of leucine present, meeting the limitation of claim 26. The reference teaches 2.78 g of methionine in 100 g of protein, meeting the limitation of at least about 0.5% to about 5% of methionine of claim 7.

The reference further teaches that the nutritional composition comprises vitamin E (tocopherol (all natural form or d1-alpha-tocopherol acetate) (see Table 6), meeting the limitation of claim 11. The reference teaches that the EPA is in the amount of 1.09 g and DHA is in the amount of 0.46 g (see Table 3), meeting the limitation of claims 8-10.

The reference further teaches that for treatment of ulcerative colitis, compositions include a protein source that can be intact or hydrolyzed proteins of high biological value (see column 3, lines 1-5) and teaches 75% whey protein concentrate as one of the ingredients (see table 7). Furthermore, since the reference teaches a whey protein concentrate, this protein would inherently comprise essential and conditionally essential amino acid profiled, thus meeting the limitation of range from about 0.60 to about 0.90 amino acids. Furthermore, the reference teaches that for example, the daily nutritional management of liver cancer includes administration of 2 to 4 containers of 8 ounces servings (237 mL) of the nutritional composition providing a daily amount of (i) combined EPA and DHA in the range of 3 to 6 g (preferred dosage 3 g), (ii) BCAA in the range of 5 to 25 g (preferred dosage about 10-15 g), (iii) vitamin C in the range of 125 to 500 mg (preferred about 300 mg), (iv) vitamin E (tocopherol) in the range of 50 to 250 IU (preferred 150 IU), (v) beta-carotene in the range of 1250 to 3250 μg (preferred 2500 μg), (vi) selenium in the range of 40 to 60 μg (preferred about 45 μg). The reference teaches that for cancer cachexia and anorexia, the effect of nutritional intervention are monitored at monthly intervals as known in the art, and depending on the results obtained, the therapeutic regimen is developed to maintain and/or boost the weight gain by the patient (see column 15, lines 44-65, Example III). The mass equivalents of 1 IU for vitamin E is 0.667 mg d-alpha-tocopherol, or of 1 mg of d1-alpha-tocopherol acetate (definition of IU from medicinenet.com). Therefore, 50 to 250 IU would equal to 33.55 mg to 167.75 mg of tocopherol, meeting the limitation of claim 13. The difference

between the reference and the instant claims is that the reference does not teach at least 30% to about 95% by weight of leucine.

16. However, Allen teaches that maintaining muscle mass while minimizing the accumulation of fat has long been an issue of concern to athletes. Food and/or vitamin supplements, as well as pituitary growth hormone, are necessary for muscle growth. Such ergogenic aids, that is supplements which stimulate muscle growth, include the three amino acids, leucine, isoleucine and valine (see paragraph [0004]). Furthermore, Phillips (1997) teaches that beta-hydroxy beta-methylbutyrate (HMB) is a metabolite of the essential amino acid leucine (see p. 66, “What is HMB?”). Phillips teaches that HMB up-regulate the ability to build muscle and burn fat, and may help decrease stress-induced muscle protein breakdown, and enhance increases in both muscle size and strength (see p. 66, “What do the scientific studies show?").

17. Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings and optimize the amount of leucine in the composition to enhance the muscle performance. One of ordinary skill in the art would have been motivated to optimize the amount of leucine in the composition, since Allen teaches that leucine, isoleucine and valine are three amino acids that stimulate muscle growth, and Phillips teaches that leucine breaks down into HMB that build muscle, burn fat, and increases in both muscle size and strengths. One would be motivated to give leucine to produce the down stream compound HMB. Again, Leu, Ile and Val are well known to be used for stimulating muscle growth. The MPEP states that "Similarly, a prima facie case of obviousness exists where the claimed ranges and prior art ranges do not overlap but

are close enough that one skilled in the art would have expected them to have the same properties. *Titanium Metals Corp of America v. Banner*, 778 F.2d 775, 227 USPQ 773 (Fed. Cir. 1985)." See MPEP 2144.05. The artisan would have expected Leu to behave the same at 25 or 30 or 35. Applicant's own example 4 shows clearly that Leu does not improve muscle building. The amount of protein synthesis and protein breakdown for both 25% leucine and 35% leucine was about the same for both leucine concentrations (see Table 4 of instant specification). Therefore, the differing concentrations of leucine would function the same. It appears that the additional components are important to the reduction of breakdown (see Table 4 of instant specification).

Furthermore, the MPEP further states: Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. *"[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)* (Claimed process which was performed at a temperature between 40°C and 80°C and an acid concentration between 25% and 70% was held to be prima facie obvious over a reference process which differed from the claims only in that the reference process was performed at a temperature of 100°C and an acid concentration of 10%.); see also Peterson, 315 F.3d at 1330, 65 USPQ2d at 1382 ("*The normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages."); In re*

<u>Hoeschele</u>, *406 F.2d 1403, 160 USPQ 809 (CCPA 1969)* (Claimed elastomeric polyurethanes which fell within the broad scope of the references were held to be unpatentable thereover because, among other reasons, <u>there was no evidence of the criticality of the claimed ranges of molecular weight or molar proportions</u>.). For more recent cases applying this principle, see Merck & Co. Inc. v. Biocraft Laboratories Inc., 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), cert. denied, 493 U.S. 975 (1989); *<u>In re Kulling</u>*, 897 F.2d 1147, 14 USPQ2d 1056 (Fed. Cir. 1990); and *<u>In re Geisler</u>*, 116 F.3d 1465, 43 USPQ2d 1362 (Fed. Cir. 1997). Knowing that leucine, isoleucine and valine stimulate muscle growth, it would have been obvious to one or ordinary skill in the art to optimize the concentrations of leucine, isoleucine or valine to enhance the muscle growth. Furthermore, since leucine produces the down stream compound of HMB that is important in muscle growth and stimulation, it would have been obvious to optimize the concentration of leucine to produce the optimal composition. From the teachings of the references, it is apparent that one of the ordinary skills in the art would have had a reasonable expectation of success in producing the claimed invention.

Thus, the invention as a whole is *prima facie* obvious over the references, especially in the absence of evidence to the contrary.

Response to Applicant's Arguments

18. Applicant argues that "Hageman, Salvati, Allen and Phillips fail to remedy the deficiencies of Abbruzzese. Hageman, Salvati, Allen and Phillips fail to disclose or suggest leucine, in free and/or salt form, is present in an amount of at least 30% by

weight based on the weight of intact protein as required by the present claims....fail to disclose or suggest a ratio of total essential amino acids and, optionally, conditionally essential amino acids to total amino acids ranging from about 0.60 to about 0.90 as required by independent claims 3, 17, 25 and 28." Applicant further argues that "Hageman, Salvati, Allen and Phillips fail to disclose or suggest wherein the ratio of leucine in free and/or salt form to leucine in form of the intact protein is about 3:1 to about 1:3 as required by independent claims 3, 17, 25 and 28." Applicant further argues that "there is absolutely no guidance in Hageman and Salvati for one of skill in the art to choose the active components and effective amount of the components present in the instant claims to achieve the unexpectedly improved composition as Applicant has…the skilled artisan would have to select these specific components from the numerous components taught by Hageman and Salvati." Applicant further argues that "the skilled artisan would not arrive at the claimed invention using Hageman and Salvati in the absence of hindsight because the cited references are entirely directed to compositions utilizing different nutritional ingredients for different intended purposes. Moreover, Hageman and Salvati fail to even recognize the surprising and unexpected benefits of the claimed compositions having optimal amounts of leucine and essential amino acids."

19. Applicant's arguments have been fully considered but have not been found persuasive. The instant claims are drawn to a composition. The claims are not drawn to method claims, and do not recite any intended use. The primary reference teaches all of the active components of instant claims. The claims only require that at least 30% to

about 95% by weight of leucine be present based on the weight of total amino acids or weight of intact protein. The prior arts meet this limitation. As described in the body of the rejection, the reference teaches a liquid nutritional composition comprising (a) at least 1000 mg per liter of ω-3 fatty acids, wherein the weight ratio of ω-6 fatty acids to ω-3 fatty acids is from about 0.1 to about 1.0; (b) <u>at least 50 grams per liter</u> of a source of amino nitrogen, wherein 15 to 50% by weight of the amino-nitrogen is branched-chain amino acids, and wherein tryptophan is present in an amount less than about 5.0% by weight of the total amino -nitrogen, and (c) at least 1 gram per liter of an antioxidant system comprising beta-carotene, vitamin C, vitamin E and selenium (see claim 1). The reference teaches that the total amount of branched-chain amino acids ("BCAA") useful in the present invention is about 15-50 g/100 g protein (i.e. percent), preferably about 15-25 g/100 g. The reference teaches that the daily delivery of BCAAs is about 5-26 g (see column 9, lines 26-31). The reference teaches the branched-chain amino acids valine, leucine, isoleucine or mixtures thereof (see abstract). Since there is 9.08 g of leucine and total of 19.75 g of BCAA, 9.08/19.75 is about 46% of the leucine in the BCAA. Since the reference teaches that the total amount of BCAA useful in the present invention is about 15-50 g/100 g protein, and there is 46% of leucine in the BCAA composition, this implies that there is about 23% of leucine.

Hageman et al teach a nutritional, pharmaceutical or dietetic preparation can be manufacture in dry form, as bar, as powder, as tablet, and cookie or as cereal (see column 5, lines 60-63). The reference teaches for products for sportsmen the following mixtures of amino acids appeared to be especially <u>beneficial for muscle growth</u>, when

consumed in an amount of more than 2 and preferably more than 4 g per daily dose: 3-10 wt% histidine, 5-15% isoleucine, 10-23% % leucine, 10-23% lysine, 5-15% methionine, 5-15 wt % phenylalanine, and 5-15 wt % threonine (see column 6, lines 59-67 and column 7, line 1). Furthermore, Salvati et al teach a fused cyclic compound and the use of the fused cyclic compound with a nutritional supplements in combination with whey protein or casein, amino acids (such as leucine, branched amino acid and hydroxymethylbutyrate), triglycerides, vitamins (e.g., A, B6, B12, folate, C, D, and E), minerals, etc (see column 45, lines 48-56). Furthermore, the reference teaches anti-proliferative agents for use in combination with the compounds such as adriamycin (see column 45, lines 41-43) and anti-cancer agents, such as methotrexate, 5-fluorouracil (see column 46, lines 64-67). The reference teaches a kit comprising a first container (such as a vial) containing a pharmaceutical formulation comprising a compound, a second container (such as a vial) containing a pharmaceutical formulation comprising one or more agents to be used in combination with the compound of the invention (see 47, lines 55-64). Allen and Phillips teach the importance of leucine, isoleucine and valine in stimulating muscle growth.

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings because all references teach nutritional compositions comprising differing amounts of protein and essential amino acids (such as leucine) for the same purpose (muscle enhancement). Salvati reference teaches combining antiproliferative agents for use in combination with the compounds and a kit containing the pharmaceutical formulation. Furthermore, the composition taught in Abbruzzese,

Hageman, and Salvati can be used for the same purpose, for administering nutritional composition for such patients as cancer patients. It would have been obvious to one of ordinary skill in the art to optimize the different amino acid concentration, especially leucine, since it is well known in the art that leucine, isoleucine and valine play am important role in muscle enhancement. The instant claims are drawn to a composition, and intended use is not recited. As evidenced by the instant specification, the compositions of the instant application are for the promotion of muscle protein synthesis and control of tumor-induced weight loss in patients that are, for example, suffering from cancer cachexia (see abstract). Due to the loss of appetite due to cancer treatment, and due to appetite suppression, nutrition is necessarily a part of treatment process for improving patient’s everyday life. For the process of improving and treating cancer and loss of appetite due to cancer, a nutritional requirement would be optimized and adjusted accordingly by those skilled in the art. Abbruzzese teaches that the therapeutic regimen will be developed to maintain and/or boost weight gain, giving motivation to optimize the nutritional content. From the teachings of the references, it is apparent that one of the ordinary skills in the art would have had a reasonable expectation of success in producing the claimed invention. Thus, the invention as a whole is *prima facie* obvious over the references, especially in the absence of evidence to the contrary. There is a reasonable expectation of success, since Salvati et al teach a kit that can comprise any agent, nutritional supplement for the treatment of cancer (prostate), and Hageman et al and Abbruzzese et al teach a nutritional supplement comprising essential amino acids that is useful in treating variety of diseases, including cancer.

Both Allen and Phillips references were utilized to show the important role leucine plays in muscle enhancement.

In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

In response to Applicant's argument that "Hageman and Salvati fail to even recognize the surprising and unexpected benefits of the claimed composition having optimal amount of leucine and essential amino acids," Applicant has not compared the results of the instant components to the components of prior arts. The specification discloses that protein synthesis in muscle in Leu (25%) was 6.8 ± 1.1; Leu(35%) was 6.9 ± 0.6; Leucine alone was 6.6 ± 0.7. Protein breakdown in Leu (25%) was 8.1 ± 1.2; Leu (35%) was 8.0 ± 0.4; Leucine alone was 9.2 ± 0.5 (see Table 4). There is not much difference between Leu (25%) and Leu (35%) and Leu alone for protein synthesis. There is not much difference between Leu (25%) and Leu (35%) for protein breakdown.

It is known in the art that that supplements which stimulate muscle growth, include the three amino acids, leucine, isoleucine and valine (see paragraph [0004] in Allen reference). Furthermore, Phillips (1997) teaches that beta-hydroxy beta-

methylbutyrate (HMB) is a metabolite of the essential amino acid leucine (see p. 66, "What is HMB?"). Phillips teaches that HMB up-regulate the ability to build muscle and burn fat, and may help decrease stress-induced muscle protein breakdown, and enhance increases in both muscle size and strength (see p. 66, "What do the scientific studies show?"). Therefore, one of ordinary skill in the art would have been motivated to optimize the concentration of different amino acids, specifically leucine, isoleucine and valine to stimulate muscle growth, to arrive at the optimal composition for the treatment of muscle enhancement for cancer patients.

New Rejection

35 U.S.C. 102

20. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

21. Claims 1, 23-25 are rejected under 35 U.S.C. 102(b) as being anticipated by Heyland et al (US Patent No. 4,544,568).

22. Heyland et al teach a composition comprising a 16 kg of technical leucine and 15 kg of whey powder (see column 5, lines 34-36). The reference teaches that technical leucine comprises a dry matter content of 99.6% and containing 65% of pure leucine,

13% of Cl^-, 18% of isoleucine and 2% of valine, the remainder consisting primarily of ash and phenylalanine (see column 5, lines 24-28). The reference teaches a composition comprising 150 g technical leucine, and 180 g whey powder (see Examples 2 and 3). This implies that per 150 g of technical leucine, there is 97.5 g of leucine, 27 g of isoleucine, 3 g of valine and about 3 g of phenylalanine. Therefore, 97.5 g leucine / 180 g whey protein gives 54.2% leucine. Therefore, the reference anticipates instant claims 1 and 23-25.

Conclusion

23. No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to JULIE HA whose telephone number is (571)272-5982. The examiner can normally be reached on Mon-Thurs, 5:30 AM to 4:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Cecilia Tsang can be reached on 571-272-0562. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Julie Ha/
Examiner, Art Unit 1654

Notice of References Cited	Application/Control No. 10/662,678	Applicant(s)/Patent Under Reexamination TROUP ET AL.	
	Examiner JULIE HA	Art Unit 1654	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-4,112,123	09-1978	Roberts, Willard Lewis	426/72
*	B	US-4,544,568	10-1985	Heyland et al.	426/650
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

EXHIBIT B

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/662,678	09/15/2003	John P. Troup	8493-US	1877

74476 7590 11/24/2010
Nestle HealthCare Nutrition
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EXAMINER	
HA, JULIE	
ART UNIT	**PAPER NUMBER**
1654	

NOTIFICATION DATE	DELIVERY MODE
11/24/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdepartment@rd.nestle.com
athena.pretory@rd.nestle.com

Office Action Summary	Application No.	Applicant(s)	
	10/662,678	TROUP ET AL.	
	Examiner	**Art Unit**	
	JULIE HA	1654	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on 13 September 2010.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-4,6-14 and 16-28 is/are pending in the application.

4a) Of the above claim(s) 6,12 and 18-22 is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-4,7-11,13,14,16,17 and 23-28 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

DETAILED ACTION

Amendment after Non-final office action filed on September 13, 2010 is acknowledged. Claims 1-4, 6-14 and 16-28 are pending in this application. Claims 6, 12 and 18-22 remain withdrawn from further consideration, as being drawn to nonelected inventions and species. Claims 1-4, 7-11, 13-14, 16-17 and 23-28 are examined on the merits in this office action. After further review, a non final office action follows below.

1. This application contains claims 6, 12 and 18-22 drawn to an invention nonelected with traverse in the reply filed on October 4, 2007. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP § 821.01.

Withdrawn Rejection

2. Rejection of claims 1, 23-25 under 35 U.S.C. 102(b) as being anticipated by Heyland et al (US Patent No. 4,544,568) is hereby withdrawn in view of Applicant's amendment. However, a new 103 rejection follows due to Applicant's amendment.

Revised and Maintained Rejection

35 U.S.C. 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the

invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

For the purpose of this invention, the level of ordinary skill in the art is deemed to be at least that level of skill demonstrated by the patents in the relevant art. Joy Technologies Inc. V. Quigg, 14 USPQ2d 1432 (DC DC 1990). One of ordinary skill in the art is held in accountable not only for specific teachings of references, but also for inferences which those skilled in the art may reasonably be expected to draw. In re Hoeschele, 160 USPQ 809, 811 (CCPA 1969). In addition, one of ordinary skill in the art is motivated by economics to depart from the prior art to reduce costs consistent with desired product properties. In re Clinton, 188 USPQ 365, 367 (CCPA 1976);In re Thompson, 192 USPQ 275, 277 (CCPA 1976).

4. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

5. (Revised due to Applicant's amendment) Claims 1-4, 7-11, 13-14, 16-17 and 23-28 remain rejected under 35 U.S.C. 103(a) as unpatentable over Abbruzzese et al (US Patent No. 6,077,828) as evidenced by Roberts (US Patent No. 4,112,123), in view of

Hageman et al (US Patent No. 6,420,342) and Salvati et al (US Patent No. 6,953,679) and Vickery (US Patent No. 6,203,820).

6. Abbruzzese et al teach nutritional compositions for the prevention of cachexia and anorexia. The reference teaches a composition comprising effective amounts of ω-3 fatty acids, such as alpha-linolenic acid, stearidonic acid, eicosapentanenoic acid, docosapentaenoic acid, docosahexanoic acid or mixtures thereof; of branched-chain amino acids valine, leucine, isoleucine or mixtures thereof; with or without reduced levels of tryptophan and 5-hydroxytryptophan; and of antioxidant system selected from the group consisting of beta-carotene, vitamin C, vitamin E, selenium or mixtures thereof (see abstract). The reference teaches a liquid nutritional composition comprising (a) at least 1000 mg per liter of ω-3 fatty acids, wherein the weight ratio of ω-6 fatty acids to ω-3 fatty acids is from about 0.1 to about 1.0; (b) at least 50 grams per liter of a source of amino nitrogen, wherein 15 to 50% by weight of the amino-nitrogen is branched-chain amino acids, and wherein tryptophan is present in an amount less than about 5.0% by weight of the total amino -nitrogen, and (c) at least 1 gram per liter of an antioxidant system comprising beta-carotene, vitamin C, vitamin E and selenium (see claim 1). The reference teaches that the total amount of branched-chain amino acids (“BCAA") useful in the present invention is about 15-50 g/100 g protein (i.e. percent), preferably about 15-25 g/100 g. Thus, an 8 oz container of the nutritional composition would contain up to about 8 g BCAAs per 16 grams of total protein. The daily delivery of BCAAs is about 5-26 g (see column 9, lines 26-31). The reference teaches the branched-chain amino acids valine, leucine, isoleucine or mixtures thereof (see

abstract). Therefore, since there is 9.08 g and total of 19.75 g of BCAA, 9.08/19.75 is about 46% of the leucine in the BCAA. Since the reference teaches that the total amount of BCAA useful in the present invention is about 15-50 g/100 g protein, and there is 46% of leucine in the BCAA composition, this implies that there is about 23% of leucine. Additionally, the reference teaches that the nutritional compositions comprises branched-chain amino acids, valine, leucine, isoleucine or mixtures of thereof. Furthermore, the reference teaches that the liquid nutritional composition comprises per liter (a) at least 0.45 gm (450 mg) of ω-3 fatty acids, (b) at least 50 grams of a source of amino-nitrogen wherein 15-50% by weight of the amino-nitrogen is branched-chain amino acids and wherein tryptophan is present in an amount less than about 5.0% of the total amino-nitrogen, and (c) at least 1 gram of an antioxidant (see column 4, lines 20-36). Therefore, if there was 46% of leucine present, and 46% of 50 gram is 23 grams of leucine present in the composition, this implies that there is about 46% of leucine present in the composition.

The reference further teaches that the composition comprises essential amino acids, such as lysine, isoleucine, methionine, phenylalanine, threonine, tryptophan, valine or histidine, and teaches the amino acid profile of a nutritional composition (see Table 4). Since there is at least about 36 g of essential and/or conditionally essential amino acids per serving and about 15-50 g of BCAA per 100 g of protein, meeting the limitation of claims 14 and 16. Furthermore, since the reference teaches that there is at least 50 grams of a source of amino-nitrogen wherein 15-50% by weight of the amino nitrogen is BCAA, and Table 4 indicates that 9.08 gram of leucine is present, according

to the calculation above, this equals about 46% of BCAA. Thus, if 50% by weight of the amino nitrogen is BCAA, there is at least 25 grams of BCAA present. And if about 46% of BCAA is leucine, this implies that there is about 11.5 g of leucine present, meeting the limitation of claim 26. The reference teaches 2.78 g of methionine in 100 g of protein, meeting the limitation of at least about 0.5% to about 5% of methionine of claim 7, The reference teaches that the nutritional composition comprises vitamin E (tocopherol (all natural form or d1-alpha-tocopherol acetate) (see Table 6), meeting the limitation of claim 11. The reference teaches that the EPA is in the amount of 1.09 g and DHA is in the amount of 0.46 g (see Table 3), meeting the limitation of claims 8-10. The reference further teaches that for treatment of ulcerative colitis, compositions include a protein source that can be intact or hydrolyzed proteins of high biological value (see column 3, lines 1-5) and teaches 75% whey protein concentrate as one of the ingredients (see table 7). Furthermore, since the reference teaches a whey protein concentrate, this protein would inherently comprise essential and conditionally essential amino acid profiled, thus meeting the limitation of range from about 0.60 to about 0.90 amino acids.

As evidenced by Roberts (US Patent No. 4,112,123), per 100 g of whey protein, there is 13.0 g of leucine (see Table 1). Since there is at least 9.08 g of leucine to 13.0 g of leucine in 100 g or whey protein, this would meet the limitation of ratio of 1:3 to 3:1. Furthermore, the reference teaches that for example, the daily nutritional management of liver cancer includes administration of 2 to 4 containers of 8 ounces servings (237 mL) of the nutritional composition providing a daily amount of (i) combined EPA and

DHA in the range of 3 to 6 g (preferred dosage 3 g), (ii) BCAA in the range of 5 to 25 g (preferred dosage about 10-15 g), (iii) vitamin C in the range of 125 to 500 mg (preferred about 300 mg), (iv) vitamin E (tocopherol) in the range of 50 to 250 IU (preferred 150 IU), (v) beta-carotene in the range of 1250 to 3250 μg (preferred 2500 μg), (vi) selenium in the range of 40 to 60 μg (preferred about 45 μg). The reference teaches that for cancer cachexia and anorexia, the effect of nutritional intervention are monitored at monthly intervals as known in the art, and depending on the results obtained, the therapeutic regimen is developed to maintain and/or boost the weight gain by the patient (see column 15, lines 44-65, Example III). The mass equivalents of 1 IU for vitamin E is 0.667 mg d-alpha-tocopherol, or of 1 mg of d1-alpha-tocopherol acetate (definition of IU from medicinenet.com). Therefore, 50 to 250 IU would equal to 33.55 mg to 167.75 mg of tocopherol, meeting the limitation of claim 13.

The difference between the reference and the instant claims is that the reference does not teach at least 30% by weight of leucine based on the weight of intact protein, valine in an amount of about 8% to about 10% by weight of total amino acids, a kit comprising a first composition and a second composition comprising an anti-cancer drug, wherein said anticancer drug is 5-fluorouracil, mitomycin-C, adriamycin, chloroethyl nitrosureas or methotrexate, and that the methionine in free and/or salt from is in an amount of at least about 5% to about 7% by weight on the weight of total amino acids.

7. However, Hageman et al teach a nutritional, pharmaceutical or dietetic preparation can be manufacture in dry form, as bar, as powder, as tablet, and cookie or

as cereal (see column 5, lines 60-63). The reference teaches for products for sportsmen the following mixtures of amino acids appeared to be especially beneficial for muscle growth, when consumed in an amount of more than 2 and preferably more than 4 g per daily dose: 3-10 wt% histidine, 5-15% isoleucine, 10-23% % leucine, 10-23% lysine, 5-15% methionine, 5-15 wt % phenylalanine, and 5-15 wt % threonine (see column 6, lines 59-67 and column 7, line 1). Furthermore, the reference teaches that when proteins are included in the nutritional preparations, the amount that is included depends on the application (see column 6, lines 39-41) and the proteins are proteins of dairy, vegetable or animal origin, such as skimmed milk powder, whey powder, egg white powder, potato protein, soy protein, etc., or hydrolysates, or mixtures thereof (see column 6, lines 27-32). The reference teaches that when proteins are included in the nutritional preparation, the amount that is included depends on the application of the product. In complete formula typically an amount of 5-120 g per daily dose…for young infants the amount will be in the range 5-15 g per daily dose...in complete enteral nutrition for feeding surgery patients, typically 50-120 g per daily dose…In supplement typically 0-60 g protein per daily dose will be included (see column 6, lines 39-50). In regards to claim 25, the claim is drawn to "a composition consisting essentially of..." In regards to claim 27, the claim is drawn to "a kit comprising: a first composition consisting essentially of..." Applicant has not defined what encompasses "consisting essentially of" in the specification. In fact the instant specification does not define the phrase "consisting essentially of". The MPEP states the following: "The transitional phrase ‘consisting essentially of’ limits the scope of a claim to the specified materials or

steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. A ‘consisting essentially of’ claim occupies the middle ground between closed claims that are written in a consisting of’ format and fully open claims that are drafted in a comprising' format...For the purposes of searching for and applying prior art under 35 U.S.C. 102 and 103, absent a clear indication in the specification or claims of what the basic and novel characteristics actually are, "consisting essentially of” will be construed as equivalent to 'comprising'" (see MPEP 2105). Therefore, claims 25 and 28 have been treated as “a composition comprising…” the same claim language as original claim 3.

8. Furthermore, Salvati et al teach a fused cyclic compound and the use of the fused cyclic compound with a nutritional supplements in combination with whey protein or casein, amino acids (such as leucine, branched amino acid and hydroxymethylbutyrate), triglycerides, vitamins (e.g., A, B6, B12, folate, C, D, and E), minerals, etc (see column 45, lines 48-56). Furthermore, the reference teaches anti-proliferative agents for use in combination with the compounds such as adriamycin (see column 45, lines 41-43) and anti-cancer agents, such as methotrexate, 5-fluorouracil (see column 46, lines 64-67). The reference teaches a kit comprising a first container (such as a vial) containing a pharmaceutical formulation comprising a compound, a second container (such as a vial) containing a pharmaceutical formulation comprising one or more agents to be used in combination with the compound of the invention (see 47, lines 55-64).

9. Additionally, Vickery et al teach a composition for enhancing protein anabolism, and nutritional composition comprising L-arginine, L-cysteine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, and L-valine (see abstract and claim 1). Vickery teaches that L-leucine is useful in for lowering blood sugar, stimulating protein synthesis in muscle and wound healing of skin and bone (see column 3, lines 51-54). Vickery teaches that L-valine aids in wound healing, muscle growth and liver diseases. L-valine is present in the composition in an amount of from about 7% to about 10% by weight, from about 8% to about 9% by weight (see column 2, lines 12-30 and column 4, lines 48-53, and claims 18 and 20-21).

10. Therefore, it would have been obvious for one of ordinary skill in the art to combine the teachings of Abbruzzese et al, Hageman et al and Salvati et al and Vickery reference to produce a kit comprising the anti-cancer agent with the nutritional composition, since all of the prior art teach nutritional composition. Salvati et al teach a kit comprising fused cyclic compound, nutritional supplement comprising leucine, whey and protein and any anti-cancer agent and Hageman et al and Abbruzzese et al and Vickery teach a nutritional composition comprising different amounts of amino acids. One of ordinary skill in the art would be motivated to combine, since Salvati et al teaches such a composition/kit. Furthermore, the composition taught in Abbruzzese, Hageman, and Salvati and Vickery can be used for the same purpose, for administering nutritional composition for such patients as cancer patients for enhancing protein anabolism and muscle enhancement. As evidenced by the instant specification, the

compositions of the instant application are for the promotion of muscle protein synthesis and control of tumor-induced weight loss in patients that are, for example, suffering from cancer cachexia (see abstract). Furthermore, one of ordinary skill in the art would have been motivated to optimize the concentrations of the leucine and methionine and valine, since "it is the normal desire of scientist or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is optimum combination of percentages". The MPEP states: Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. *"[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)* (Claimed process which was performed at a temperature between 40°C and 80°C and an acid concentration between 25% and 70% was held to be prima facie obvious over a reference process which differed from the claims only in that the reference process was performed at a temperature of 100°C and an acid concentration of 10%.); see also Peterson, 315 F.3d at 1330, 65 USPQ2d at 1382 ("*The normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages."); In re Hoeschele, 406 F.2d 1403, 160 USPQ 809 (CCPA 1969)* (Claimed elastomeric polyurethanes which fell within the broad scope of the references were held to be unpatentable thereover because, among other reasons,

there was no evidence of the criticality of the claimed ranges of molecular weight or molar proportions.). For more recent cases applying this principle, see Merck & Co. Inc. v. Biocraft Laboratories Inc., 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), cert. denied, 493 U.S. 975 (1989); _In re Kulling_, 897 F.2d 1147, 14 USPQ2d 1056 (Fed. Cir. 1990); and _In re Geisler_, 116 F.3d 1465, 43 USPQ2d 1362 (Fed. Cir. 1997). Furthermore, Abbruzzese patent '828 teaches that the effect of nutritional intervention on cancer cachexia and anorexia are monitored as known in the art, and depending on the results obtained, the therapeutic regimen is developed to maintain and /or boost weight gain by the patient, with the ultimate goal of achieving tumor regression and complete eradication of cancer cells (see column 15, lines 57-64). Therefore, there is a reasonable expectation of success to optimize the concentrations of the essential amino acid/ leucine/ methionine, since it is "the normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages" and one of ordinary skill in the art would experiment with different concentrations to produce the optimal product. Due to the loss of appetite due to cancer treatment, and due to appetite suppression, nutrition is necessarily a part of treatment process for improving patient's everyday life. For the process of improving and treating cancer and loss of appetite due to cancer, a nutritional requirement would be optimized and adjusted accordingly by those skilled in the art. Abbruzzese teaches that the therapeutic regimen will be developed to maintain and/or boost weight gain, giving motivation to optimize the nutritional content. From the teachings of the references, it is

apparent that one of the ordinary skills in the art would have had a reasonable expectation of success in producing the claimed invention. Thus, the invention as a whole is *prima facie* obvious over the references, especially in the absence of evidence to the contrary. There is a reasonable expectation of success, since Salvati et al teach a kit that can comprise any agent, nutritional supplement for the treatment of cancer (prostate), and Hageman et al and Abbruzzese et al teach a nutritional supplement comprising essential amino acids that is useful in treating variety of diseases, including cancer, and Vickery teaches a nutritional composition comprising essential amino acid, including leucine, valine, isoleucine and methionine to enhance protein anabolism and enhancing muscle growth and protein synthesis in the muscle.

11. (Revised due to amendment to the claims) Claims 1-4, 7-11, 13-14, 16 and 23-26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Abbruzzese et al (US Patent No. 6,077,828, filed with IDS) as evidenced by Roberts (US Patent No. 4,112,123), in view of Allen et al (US 2003/0119888 A1) and Phillips Bill (Sports Supplement Review, 1997, pp. 66-70) and Vickery (US Patent No. 6,203,820).

12. Abbruzzese et al teach nutritional compositions for the prevention of cachexia and anorexia. The reference teaches a composition comprising effective amounts of ω-3 fatty acids, such as alpha-linolenic acid, stearidonic acid, eicosapentanenoic acid, docosapentaenoic acid, docosahexanoic acid or mixtures thereof; of branched-chain amino acids valine, leucine, isoleucine or mixtures thereof; with or without reduced levels of tryptophan and 5-hydroxytryptophan; and of antioxidant system selected from

the group consisting of beta-carotene, vitamin C, vitamin E, selenium or mixtures thereof (see abstract). The reference teaches a liquid nutritional composition comprising (a) at least 1000 mg per liter of ω-3 fatty acids, wherein the weight ratio of ω-6 fatty acids to ω-3 fatty acids is from about 0.1 to about 1.0; (b) at least 50 grams per liter of a source of amino nitrogen, wherein 15 to 50% by weight of the amino-nitrogen is branched-chain amino acids, and wherein tryptophan is present in an amount less than about 5.0% by weight of the total amino -nitrogen, and (c) at least 1 gram per liter of an antioxidant system comprising beta-carotene, vitamin C, vitamin E and selenium (see claim 1). The reference teaches that the total amount of branched-chain amino acids (“BCAA") useful in the present invention is about 15-50 g/100 g protein (i.e. percent), preferably about 15-25 g/100 g. Thus, an 8 oz container of the nutritional composition would contain up to about 8 g BCAAs per 16 grams of total protein. The daily delivery of BCAAs is about 5-26 g (see column 9, lines 26-31). The reference teaches the branched-chain amino acids valine, leucine, isoleucine or mixtures thereof (see abstract). Therefore, since there is 9.08 g and total of 19.75 g of BCAA, 9.08/19.75 is about 46% of the leucine in the BCAA. Since the reference teaches that the total amount of BCAA useful in the present invention is about 15-50 g/100 g protein, and there is 46% of leucine in the BCAA composition, this implies that there is at least about 23% of leucine. Additionally, the reference teaches that the nutritional compositions comprises branched-chain amino acids, valine, leucine, isoleucine or mixtures of thereof. As evidenced by Roberts (US Patent No. 4,112,123), per 100 g of whey protein,

there is 13.0 g of leucine (see Table 1). Since there is at least 9.08 g of leucine to 13.0 g of leucine in 100 g or whey protein, this would meet the limitation of ratio of 1:3 to 3:1.

Furthermore, the reference teaches that the liquid nutritional composition comprises per liter (a) at least 0.45 gm (450 mg) of ω-3 fatty acids, (b) at least 50 grams of a source of amino-nitrogen wherein 15-50% by weight of the amino-nitrogen is branched-chain amino acids and wherein tryptophan is present in an amount less than about 5.0% of the total amino-nitrogen, and (c) at least 1 gram of an antioxidant (see column 4, lines 20-36). Therefore, if there was 46% of leucine present, and 46% of 50 gram is 23 grams of leucine present in the composition, this implies that there is about 46% of leucine present in the composition.

The reference further teaches that the composition comprises essential amino acids, such as lysine, isoleucine, methionine, phenylalanine, threonine, tryptophan, valine or histidine, and teaches the amino acid profile of a nutritional composition (see Table 4). Since there is at least about 36 g of essential and/or conditionally essential amino acids per serving and about 15-50 g of BCAA per 100 g of protein, meeting the limitation of claims 14 and 16. Furthermore, since the reference teaches that there is at least 50 grams of a source of amino-nitrogen wherein 15-50% by weight of the amino nitrogen is BCAA, and Table 4 indicates that 9.08 gram of leucine is present, according to the calculation above, this equals about 46% of BCAA. Thus, if 50% by weight of the amino nitrogen is BCAA, there is at least 25 grams of BCAA present. And if about 46% of BCAA is leucine, this implies that there is about 11.5 g of leucine present, meeting the limitation of claim 26. The reference teaches 2.78 g of methionine in 100 g of

protein, meeting the limitation of at least about 0.5% to about 5% of methionine of claim 7.

The reference further teaches that the nutritional composition comprises vitamin E (tocopherol (all natural form or d1-alpha-tocopherol acetate) (see Table 6), meeting the limitation of claim 11. The reference teaches that the EPA is in the amount of 1.09 g and DHA is in the amount of 0.46 g (see Table 3), meeting the limitation of claims 8-10. The reference further teaches that for treatment of ulcerative colitis, compositions include a protein source that can be intact or hydrolyzed proteins of high biological value (see column 3, lines 1-5) and teaches 75% whey protein concentrate as one of the ingredients (see table 7). Furthermore, since the reference teaches a whey protein concentrate, this protein would inherently comprise essential and conditionally essential amino acid profiled, thus meeting the limitation of range from about 0.60 to about 0.90 amino acids. Furthermore, the reference teaches that for example, the daily nutritional management of liver cancer includes administration of 2 to 4 containers of 8 ounces servings (237 mL) of the nutritional composition providing a daily amount of (i) combined EPA and DHA in the range of 3 to 6 g (preferred dosage 3 g), (ii) BCAA in the range of 5 to 25 g (preferred dosage about 10-15 g), (iii) vitamin C in the range of 125 to 500 mg (preferred about 300 mg), (iv) vitamin E (tocopherol) in the range of 50 to 250 IU (preferred 150 IU), (v) beta-carotene in the range of 1250 to 3250 μg (preferred 2500 μg), (vi) selenium in the range of 40 to 60 μg (preferred about 45 μg). The reference teaches that for cancer cachexia and anorexia, the effect of nutritional intervention are monitored at monthly intervals as known in the art, and depending on the results

obtained, the therapeutic regimen is developed to maintain and/or boost the weight gain by the patient (see column 15, lines 44-65, Example III). The mass equivalents of 1 IU for vitamin E is 0.667 mg d-alpha-tocopherol, or of 1 mg of d1-alpha-tocopherol acetate (definition of IU from medicinenet.com). Therefore, 50 to 250 IU would equal to 33.55 mg to 167.75 mg of tocopherol, meeting the limitation of claim 13. The difference between the reference and the instant claims is that the reference does not teach at least 30% to about 95% by weight of leucine and about 8% to about 10% valine.

13. However, Allen teaches that maintaining muscle mass while minimizing the accumulation of fat has long been an issue of concern to athletes. Food and/or vitamin supplements, as well as pituitary growth hormone, are necessary for muscle growth. Such ergogenic aids, that is supplements which stimulate muscle growth, include the three amino acids, leucine, isoleucine and valine (see paragraph [0004]). Furthermore, Phillips (1997) teaches that beta-hydroxy beta-methylbutyrate (HMB) is a metabolite of the essential amino acid leucine (see p. 66, “What is HMB?”). Phillips teaches that HMB up-regulate the ability to build muscle and burn fat, and may help decrease stress-induced muscle protein breakdown, and enhance increases in both muscle size and strength (see p. 66, “What do the scientific studies show?").

14. Additionally, Vickery et al teach a composition for enhancing protein anabolism, and nutritional composition comprising L-arginine, L-cysteine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, and L-valine (see abstract and claim 1). Vickery teaches that L-leucine is useful in for lowering blood sugar, stimulating protein synthesis in muscle and wound

healing of skin and bone (see column 3, lines 51-54). Vickery teaches that L-valine aids in wound healing, muscle growth and liver diseases. L-valine is present in the composition in an amount of from about 7% to about 10% by weight, from about 8% to about 9% by weight (see column 2, lines 12-30 and column 4, lines 48-53, and claims 18 and 20-21).

15. Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings and optimize the amount of leucine and valine in the composition to enhance the muscle performance. One of ordinary skill in the art would have been motivated to optimize the amount of leucine and valine in the composition, since Allen teaches that leucine, isoleucine and valine are three amino acids that stimulate muscle growth, Phillips teaches that leucine breaks down into HMB that build muscle, burn fat, and increases in both muscle size and strengths, and Vickery teaches that L-leucine is useful in for lowering blood sugar, stimulating protein synthesis in muscle and wound healing of skin and bone. Vickery teaches that L-valine aids in wound healing, muscle growth and liver diseases. L-valine is present in the composition in an amount of from about 7% to about 10% by weight, from about 8% to about 9% by weight. One would be motivated to give leucine to produce the down stream compound HMB and valine to aid in muscle growth. Again, Leu, Ile and Val are well known to be used for stimulating muscle growth. The MPEP states that "Similarly, a prima facie case of obviousness exists where the claimed ranges and prior art ranges do not overlap but are close enough that one skilled in the art would have expected them to have the same properties. *Titanium Metals Corp of America v. Banner*, 778 F.2d 775, 227 USPQ 773

(Fed. Cir. 1985)." See MPEP 2144.05. The artisan would have expected Leu to behave the same at 25 or 30 or 35. Applicant's own example 4 shows clearly that Leu does not improve muscle building. The amount of protein synthesis and protein breakdown for both 25% leucine and 35% leucine was about the same for both leucine concentrations (see Table 4 of instant specification). Therefore, the differing concentrations of leucine would function the same. It appears that the additional components are important to the reduction of breakdown (see Table 4 of instant specification).

Furthermore, the MPEP further states: Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. *"[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)* (Claimed process which was performed at a temperature between 40°C and 80°C and an acid concentration between 25% and 70% was held to be prima facie obvious over a reference process which differed from the claims only in that the reference process was performed at a temperature of 100°C and an acid concentration of 10%.); see also Peterson, 315 F.3d at 1330, 65 USPQ2d at 1382 ("*The normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages.*"); *In re Hoeschele, 406 F.2d 1403, 160 USPQ 809 (CCPA 1969)* (Claimed elastomeric polyurethanes which fell within the broad scope of the references were held to be

unpatentable thereover because, among other reasons, there was no evidence of the criticality of the claimed ranges of molecular weight or molar proportions.). For more recent cases applying this principle, see Merck & Co. Inc. v. Biocraft Laboratories Inc., 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), cert. denied, 493 U.S. 975 (1989); *In re Kulling*, 897 F.2d 1147, 14 USPQ2d 1056 (Fed. Cir. 1990); and *In re Geisler*, 116 F.3d 1465, 43 USPQ2d 1362 (Fed. Cir. 1997). Knowing that leucine, isoleucine and valine stimulate muscle growth, it would have been obvious to one or ordinary skill in the art to optimize the concentrations of leucine, isoleucine or valine to enhance the muscle growth. Furthermore, since leucine produces the down stream compound of HMB that is important in muscle growth and stimulation, it would have been obvious to optimize the concentration of leucine to produce the optimal composition. From the teachings of the references, it is apparent that one of the ordinary skills in the art would have had a reasonable expectation of success in producing the claimed invention.

Thus, the invention as a whole is *prima facie* obvious over the references, especially in the absence of evidence to the contrary.

Response to Applicant's Arguments

16. Applicant argues that "Applicant has surprisingly found that a formulation containing free essential amino acids as compared to a formulation containing free essential and non-essential amino acids or intact protein is optimal…In addition, Applicant has surprisingly and unexpectedly found that particularly useful compositions for promotion of muscle protein synthesis or controlling tumor-induced weight loss, such

as cachexia, e.g. cancer cachexia may be obtained by combining essential amino acids in free form and/or in salt form with intact protein." Applicant argues that "Abbruzzese, Roberts, Hageman, Salvati, Allen and Phillips fail to disclose or suggest compositions having leucine, valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least one essential amino acid selected from histidine, and combinations thereof in free and/or salt from, wherein said leucine, in free and/or salt form are required." Applicant argues that “Hageman discloses products having the following mixture of amino acids as beneficial for muscle growth when consumes in an amount more than 2 and preferably more than 4 g per daily dose: 3-10 wt % histidine, 5-15 wt % isoleucine, 10-23% leucine, 10-23% lysine, 5-15 wt % methionine, 5-15 wt % phenylalanine, 5-15 wt % threonine." Applicant argues that “At no place in the disclosure does Salvati, Roberts, Allen and Phillips disclose or suggest compositions containing about 8% to about 10% of valine, as required, in part, by the present claims.”

17. Applicant’s arguments have been fully considered but have not been found persuasive. The instant claims are drawn to a composition. The claims are not drawn to method claims, and do not recite any intended use. The primary reference teaches all of the active components of instant claims. The claims only require that valine in an amount of about 8% to about 10% by weight based on the weight of total amino acids, and at least 30% to about 95% by weight of leucine be present based on the weight of total amino acids or weight of intact protein. The prior arts meet this limitation. As described in the body of the rejection, the reference teaches a liquid nutritional

composition comprising (a) at least 1000 mg per liter of ω-3 fatty acids, wherein the weight ratio of ω-6 fatty acids to ω-3 fatty acids is from about 0.1 to about 1.0; (b) at least 50 grams per liter of a source of amino nitrogen, wherein 15 to 50% by weight of the amino-nitrogen is branched-chain amino acids, and wherein tryptophan is present in an amount less than about 5.0% by weight of the total amino -nitrogen, and (c) at least 1 gram per liter of an antioxidant system comprising beta-carotene, vitamin C, vitamin E and selenium (see claim 1). The reference teaches that the total amount of branched-chain amino acids ("BCAA") useful in the present invention is about 15-50 g/100 g protein (i.e. percent), preferably about 15-25 g/100 g. The reference teaches that the daily delivery of BCAAs is about 5-26 g (see column 9, lines 26-31). The reference teaches the branched-chain amino acids valine, leucine, isoleucine or mixtures thereof (see abstract). Since there is 9.08 g of leucine and total of 19.75 g of BCAA, 9.08/19.75 is about 46% of the leucine in the BCAA. Since the reference teaches that the total amount of BCAA useful in the present invention is about 15-50 g/100 g protein, and there is 46% of leucine in the BCAA composition, this implies that there is about 23% of leucine.

Hageman et al teach a nutritional, pharmaceutical or dietetic preparation can be manufacture in dry form, as bar, as powder, as tablet, and cookie or as cereal (see column 5, lines 60-63). The reference teaches for products for sportsmen the following mixtures of amino acids appeared to be especially beneficial for muscle growth, when consumed in an amount of more than 2 and preferably more than 4 g per daily dose: 3-10 wt% histidine, 5-15% isoleucine, 10-23% % leucine, 10-23% lysine, 5-15%

methionine, 5-15 wt % phenylalanine, and 5-15 wt % threonine (see column 6, lines 59-67 and column 7, line 1). Furthermore, Salvati et al teach a fused cyclic compound and the use of the fused cyclic compound with a nutritional supplements in combination with whey protein or casein, amino acids (such as leucine, branched amino acid and hydroxymethylbutyrate), triglycerides, vitamins (e.g., A, B6, B12, folate, C, D, and E), minerals, etc (see column 45, lines 48-56). Furthermore, the reference teaches anti-proliferative agents for use in combination with the compounds such as adriamycin (see column 45, lines 41-43) and anti-cancer agents, such as methotrexate, 5-fluorouracil (see column 46, lines 64-67). The reference teaches a kit comprising a first container (such as a vial) containing a pharmaceutical formulation comprising a compound, a second container (such as a vial) containing a pharmaceutical formulation comprising one or more agents to be used in combination with the compound of the invention (see 47, lines 55-64). Allen and Phillips teach the importance of leucine, isoleucine and valine in stimulating muscle growth. Vickery teaches that L-leucine is useful in for lowering blood sugar, stimulating protein synthesis in muscle and wound healing of skin and bone. Vickery teaches that L-valine aids in wound healing, muscle growth and liver diseases. L-valine is present in the composition in an amount of from about 7% to about 10% by weight, from about 8% to about 9% by weight.

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings because all references teach nutritional compositions comprising differing amounts of protein and essential amino acids (such as leucine) for the same purpose (muscle enhancement). Salvati reference teaches combining antiproliferative

agents for use in combination with the compounds and a kit containing the pharmaceutical formulation. Furthermore, the composition taught in Abbruzzese, Hageman, and Salvati can be used for the same purpose, for administering nutritional composition for such patients as cancer patients. Vickery reference teaches a nutritional composition and composition for enhancing protein anabolism. It would have been obvious to one of ordinary skill in the art to optimize the different amino acid concentration, especially leucine, since it is well known in the art that leucine, isoleucine and valine play am important role in muscle enhancement. The instant claims are drawn to a composition, and intended use is not recited. As evidenced by the instant specification, the compositions of the instant application are for the promotion of muscle protein synthesis and control of tumor-induced weight loss in patients that are, for example, suffering from cancer cachexia (see abstract). Due to the loss of appetite due to cancer treatment, and due to appetite suppression, nutrition is necessarily a part of treatment process for improving patient’s everyday life. For the process of improving and treating cancer and loss of appetite due to cancer, a nutritional requirement would be optimized and adjusted accordingly by those skilled in the art. Abbruzzese teaches that the therapeutic regimen will be developed to maintain and/or boost weight gain, giving motivation to optimize the nutritional content. From the teachings of the references, it is apparent that one of the ordinary skills in the art would have had a reasonable expectation of success in producing the claimed invention. Thus, the invention as a whole is *prima facie* obvious over the references, especially in the absence of evidence to the contrary. There is a reasonable expectation of success,

since Salvati et al teach a kit that can comprise any agent, nutritional supplement for the treatment of cancer (prostate), and Hageman et al and Abbruzzese et al teach a nutritional supplement comprising essential amino acids that is useful in treating variety of diseases, including cancer. Both Allen and Phillips references were utilized to show the important role leucine plays in muscle enhancement. Vickery reference teaches the importance of L-valine and the composition comprising about 7 to about 10% leucine along with other amino acids.

In response to Applicant's argument regarding "surprising and unexpected benefits of the claimed composition having optimal amount of leucine and essential amino acids," Applicant has not compared the results of the instant components to the components of prior arts. The specification discloses that protein synthesis in muscle in Leu (25%) was 6.8 ± 1.1; Leu(35%) was 6.9 ± 0.6; Leucine alone was 6.6 ± 0.7. Protein breakdown in Leu (25%) was 8.1 ± 1.2; Leu (35%) was 8.0 ± 0.4; Leucine alone was 9.2 ± 0.5 (see Table 4). There is not much difference between Leu (25%) and Leu (35%) and Leu alone for protein synthesis. There is not much difference between Leu (25%) and Leu (35%) for protein breakdown.

It is known in the art that that supplements which stimulate muscle growth, include the three amino acids, leucine, isoleucine and valine (see paragraph [0004] in Allen reference). Furthermore, Phillips (1997) teaches that beta-hydroxy beta-methylbutyrate (HMB) is a metabolite of the essential amino acid leucine (see p. 66, "What is HMB?"). Phillips teaches that HMB up-regulate the ability to build muscle and burn fat, and may help decrease stress-induced muscle protein breakdown, and

enhance increases in both muscle size and strength (see p. 66, "What do the scientific studies show?"). Therefore, one of ordinary skill in the art would have been motivated to optimize the concentration of different amino acids, specifically leucine, isoleucine and valine to stimulate muscle growth, to arrive at the optimal composition for the treatment of muscle enhancement for cancer patients.

New Rejection

35 U.S.C. 103

18. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

For the purpose of this invention, the level of ordinary skill in the art is deemed to be at least that level of skill demonstrated by the patents in the relevant art. Joy Technologies Inc. V. Quigg, 14 USPQ2d 1432 (DC DC 1990). One of ordinary skill in the art is held in accountable not only for specific teachings of references, but also for inferences which those skilled in the art may reasonably be expected to draw. In re Hoeschele, 160 USPQ 809, 811 (CCPA 1969). In addition, one of ordinary skill in the art is motivated

by economics to depart from the prior art to reduce costs consistent with desired product properties. *In re Clinton*, 188 USPQ 365, 367 (CCPA 1976);*In re Thompson*, 192 USPQ 275, 277 (CCPA 1976).

19. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

20. Claims 1, 23-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Heyland et al (US Patent No. 4,544,568) in view of Vickery (US Patent No. 6,203,820).

21. Heyland et al teach a composition comprising a 16 kg of technical leucine and 15 kg of whey powder (see column 5, lines 34-36). The reference teaches that technical leucine comprises a dry matter content of 99.6% and containing 65% of pure leucine, 13% of Cl^-, 18% of isoleucine and 2% of valine, the remainder consisting primarily of ash and phenylalanine (see column 5, lines 24-28). The reference teaches a composition comprising 150 g technical leucine, and 180 g whey powder (see Examples 2 and 3). This implies that per 150 g of technical leucine, there is 97.5 g of leucine, 27 g of isoleucine, 3 g of valine and about 3 g of phenylalanine. Therefore, 97.5 g leucine / 180 g whey protein gives 54.2% leucine. The difference between the reference and the instant claims is that the reference does not teach about 8% to about 10% valine.

22. However, Vickery et al teach a composition for enhancing protein anabolism, and nutritional composition comprising L-arginine, L-cysteine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, and L-valine (see abstract and claim 1). Vickery teaches that L-leucine is useful in for lowering blood sugar, stimulating protein synthesis in muscle and wound healing of skin and bone (see column 3, lines 51-54). Vickery teaches that L-valine aids in wound healing, muscle growth and liver diseases. L-valine is present in the composition in an amount of from about 7% to about 10% by weight, from about 8% to about 9% by weight (see column 2, lines 12-30 and column 4, lines 48-53, and claims 18 and 20-21).

23. Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of the prior arts because all references teach nutritional compositions comprising differing amounts of protein and essential amino acids (such as leucine) for the same purpose (muscle enhancement). One of ordinary skill in the art would be motivated to optimize the amounts of valine and isoleucine, since Vickery teaches that L-valine aids in wound healing, muscle growth and L-leucine is important in lowering blood sugar, stimulating protein synthesis in muscle and wound healing of skin and bond. Leu, Ile and Val are well known to be used for stimulating muscle growth. Vickery teaches about 7% to about 10% of leucine along with other amino acid compositions.

Furthermore, the MPEP further states: Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is <u>evidence indicating such concentration or temperature is critical</u>.

"[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)* (Claimed process which was performed at a temperature between 40°C and 80°C and an acid concentration between 25% and 70% was held to be prima facie obvious over a reference process which differed from the claims only in that the reference process was performed at a temperature of 100°C and an acid concentration of 10%.); see also Peterson, 315 F.3d at 1330, 65 USPQ2d at 1382 ("*The normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages."); In re Hoeschele, 406 F.2d 1403, 160 USPQ 809 (CCPA 1969)* (Claimed elastomeric polyurethanes which fell within the broad scope of the references were held to be unpatentable thereover because, among other reasons, there was no evidence of the criticality of the claimed ranges of molecular weight or molar proportions.). For more recent cases applying this principle, see Merck & Co. Inc. v. Biocraft Laboratories Inc., 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), cert. denied, 493 U.S. 975 (1989); *In re Kulling*, 897 F.2d 1147, 14 USPQ2d 1056 (Fed. Cir. 1990); and *In re Geisler*, 116 F.3d 1465, 43 USPQ2d 1362 (Fed. Cir. 1997). Knowing that leucine, isoleucine and valine stimulate muscle growth, it would have been obvious to one or ordinary skill in the art to optimize the concentrations of leucine, isoleucine or valine to enhance the muscle growth. From the teachings of the references, it is apparent that one of the ordinary

skills in the art would have had a reasonable expectation of success in producing the claimed invention.

Thus, the invention as a whole is *prima facie* obvious over the references, especially in the absence of evidence to the contrary.

Conclusion

24. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a). No claim is allowed.

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to JULIE HA whose telephone number is (571)272-5982. The examiner can normally be reached on Mon-Thurs, 5:30 AM to 4:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Cecilia Tsang can be reached on 571-272-0562. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Julie Ha/
Primary Examiner, Art Unit 1654

Notice of References Cited	Application/Control No. 10/662,678	Applicant(s)/Patent Under Reexamination TROUP ET AL.	
	Examiner JULIE HA	Art Unit 1654	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-6,203,820	03-2001	Vickery, Brice E.	424/646
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

EXHIBIT C

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/662,678	09/15/2003	John P. Troup	8493-US	1877

74476 7590 02/17/2011

Nestle HealthCare Nutrition
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EXAMINER	
HA, JULIE	
ART UNIT	**PAPER NUMBER**
1654	

NOTIFICATION DATE	DELIVERY MODE
02/17/2011	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdepartment@rd.nestle.com
athena.pretory@rd.nestle.com

Advisory Action Before the Filing of an Appeal Brief	Application No. 10/662,678	Applicant(s) TROUP ET AL.	
	Examiner JULIE HA	Art Unit 1654	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 10 January 2011 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

1. ☒ The reply was filed after a final rejection, but prior to or on the same day as filing a Notice of Appeal. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. The reply must be filed within one of the following time periods:
   a) ☐ The period for reply expires ______months from the mailing date of the final rejection.
   b) ☒ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.
   Examiner Note: If box 1 is checked, check either box (a) or (b). ONLY CHECK BOX (b) WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☐ The proposed amendment(s) filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   (a) ☐ They raise new issues that would require further consideration and/or search (see NOTE below);
   (b) ☐ They raise the issue of new matter (see NOTE below);
   (c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   (d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.
   NOTE: _____. (See 37 CFR 1.116 and 41.33(a)).
4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____.
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☐ For purposes of appeal, the proposed amendment(s): a) ☐ will not be entered, or b) ☐ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.
   The status of the claim(s) is (or will be) as follows:
   Claim(s) allowed: _____.
   Claim(s) objected to: _____.
   Claim(s) rejected: _____.
   Claim(s) withdrawn from consideration: _____.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ The affidavit or other evidence filed after a final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
9. ☐ The affidavit or other evidence filed after the date of filing a Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing a good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
10. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

11. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: Please see continuation of 11 below.
12. ☐ Note the attached Information *Disclosure Statement*(s). (PTO/SB/08) Paper No(s). _____
13. ☐ Other: _____.

/Julie Ha/
Primary Examiner, Art Unit 1654

Continuation of 11:

Claims 1-4, 7-11, 13-14, 16-17 and 23-28 remain rejected under 35 U.S.C. 103(a) as unpatentable over Abbruzzese et al (US Patent No. 6,077,828) as evidenced by Roberts (US Patent No. 4,112,123), in view of Hageman et al (US Patent No. 6,420,342) and Salvati et al (US Patent No. 6,953,679) and Vickery (US Patent No. 6,203,820) as set forth in the previous office action.

Applicant argues that "Independent claims 1-3, 17, 23-25 and 28 recite…compositions having leucine, valine in an amout of about 8% to about 10% by weight based on the weight of total amino acids…" Applicant argues that "At no place in the disclosure does Roberts disclose or suggest compositions containing about 8% to about 10% of valine as required, in part, by the present claims."

Applicant's arguments have been fully considered but have not been found persuasive. As indicated in the previous office action, Vickery et al teach a composition for enhancing protein anabolism and nutritional composition comprising L-arginine, L-cysteine, L-histidien, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, and L-valine. Vickery teaches a nutritional composition present in an amount of from about 7% to about 10% by weight, from about 8% to about 9% by weight. Therefore, it would have been obvious for one of ordinary skill in the art to combine the teachings of Abbruzzese et al, Hageman et al, Salvati et al and Vickery reference to produce a kit comprising the anti-cancer agent with the nutritional compoition, since all of the prior art teach nutritional composition. One of ordinary skill in the art would be motivated to to optimize the amount of leucine and valine in the nutritional composition, since it is known in the art that leucine is useful in lowering blood sugar, stimulating protein synthesis in muscle and wound healing of skin and bone, and valine aids in wound healing, muscle growth and liver diseases.

Claims 1-4, 7-11, 13-14, 16 and 23-26 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Abbruzzese et al (US Patent No. 6,077,828) as evidenced by Roberts (US Patent No. 4,112,123), in view of Allen et al (US 2003/0119888) and Phillips Bill (Sports Supplement Review) and Vickery (US Patent No. 6,203,820) as set forth in the previous office action.

Applicant argues that "Abbruzzese, Roberts, Hageman, Salvati, Allen, Phillips and Vickery all fail to disclose or suggest compositions having leucine, valine in an amoutn of about 8% to about 10% by weight based on the weight of total amino acids…"

Applicant's arguments have been fully considered but have not been found persuasive. Each of the cited references teach nutritional composition comprising the active components of instant claims. Each of the references teach why each component is important in nutritional composition. As indicated in the previous office action, Vickery et al teach a composition for enhancing protein anabolism and nutritional composition comprising L-arginine, L-cysteine, L-histidien, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, and L-valine. Vickery teaches a nutritional composition present in an amount of from about 7% to about 10% by weight, from about 8% to about 9% by weight. Therefore, it would have been obvious for one of ordinary skill in the art to combine the teachings of Abbruzzese et al, Allen et al, Phillips and Vickery reference because all references teach nutritional compositions comprising differing amounts of protein and essential amino acids (such as leucine) for the same purpose (muscle enhancement). One of ordinary skill in the art would be motivated to to optimize the amount of leucine, isoleucine and valine in the nutritional composition, since it is known in the art that leucine, isoleucine and valine play an important role in muclse enhancement. Both Allen and Phillips references were utilized to show the important role leucine plays in muscle enhancement. Vickey teaches that leucine is useful in lowering blood sugar, stimulating protein synthesis in muscle and wound healing of skin and bone, and valine aids in wound healing, muscle growth and liver diseases. Therefore, one of ordianry skill in the art would have been motivated to optimize the concentrations of different amino acids, espeically leucine, isoleucine and valine to stimulate muslce growth, to arrive at the optimal composition for the treatment of muscle enhancement for cancer patients.

Claims 1, 23-25 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Heyland et al (US Patent No. 4,544,568) in view of Vickery (US Patent No. 6,203,820) as set forth in the previous office action.

Applicant did not respond to this rejection.

The rejection is maintained as set forth in the previous office action.

EXHIBIT D

US006077828A

United States Patent [19]

Abbruzzese et al.

[11] **Patent Number: 6,077,828**

[45] **Date of Patent: *Jun. 20, 2000**

[54] **METHOD FOR THE PREVENTION AND TREATMENT OF CACHEXIA AND ANOREXIA**

[75] Inventors: **Bonnie Chandler Abbruzzese**, Dublin; **Mark Anthony McCamish**; **Frederick Oliver Cope**, both of Worthington; **Stephen Joseph Demichele**, Dublin, all of Ohio

[73] Assignee: **Abbott Laboratories**, Abbott Park, Ill.

[*] Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

[21] Appl. No.: **08/842,454**

[22] Filed: **Apr. 24, 1997**

Related U.S. Application Data

[63] Continuation-in-part of application No. 08/635,179, Apr. 25, 1996, abandoned.

[51] **Int. Cl.**^{7} **A23J 3/16**; A61K 38/17

[52] **U.S. Cl.** **514/21**; 514/578; 514/725; 514/730; 514/739; 424/523; 426/72; 426/601; 426/602; 426/656; 426/800; 426/801

[58] **Field of Search** 424/523; 514/578, 514/725, 730, 739, 21; 426/72, 601, 602, 656, 800, 801

[56] **References Cited**

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(List continued on next page.)

Primary Examiner—Ceila Chang
Attorney, Agent, or Firm—J. Michael Dixon; Thomas D. Brainard

[57] **ABSTRACT**

The present invention relates to methods and nutritional compositions for the prevention and treatment of cachexia and anorexia. The methods of the invention comprise administering a composition comprising effective amounts of ω-3 fatty acids such as alpha-linolenic acid, stearidonic acid, eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid or mixtures thereof; of branched-chain amino acids valine, leucine, isoleucine or mixtures thereof; with or without reduced levels of tryptophan and 5-hydroxytryptophan; and of antioxidant system selected from the group comprising beta-carotene, vitamin C, vitamin E, selenium, or mixtures thereof.

20 Claims, No Drawings

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METHOD FOR THE PREVENTION AND TREATMENT OF CACHEXIA AND ANOREXIA

This application is a continuation-in-part of prior application Ser. No. 08/635,179 filed Apr. 25, 1996, now abandoned which is hereby incorporated by reference.

The present invention relates to methods and nutritional compositions for the prevention and treatment of cancer cachexia and anorexia. In the practice of the present invention patients are enterally administered ω-3 fatty acids including, but not limited to alpha-linolenic (18:3 ω-3), stearidonic (18:4 ω-3), eicosapentaenoic (20:5 ω-3), docosapentaenoic (22:5 ω-3), and docosahexaenoic (22:6 ω-3), in combination with antioxidants including, but not limited to, beta-carotene, vitamin C, vitamin E, selenium, or mixtures thereof; a source of amino-nitrogen with high levels of branched-chain amino acids including valine, leucine, isoleucine, and with or without reduced levels of tryptophan and 5-hydroxytryptophan.

BACKGROUND

Cancer cachexia is a syndrome characterized by anorexia, weight loss, premature satiety, asthenia, loss of lean body mass, and multiple organ dysfunction. The majority of patients with cancer whose disease progresses to metastatic disease develop cachexia during their treatment program and the cachexia contributes to their deaths. The frequency of weight loss in cancer patients ranges from 40% for patients with breast cancer, acute myelocytic leukemia, and sarcoma to more than 80% in patients with carcinoma of the pancreas and stomach. About 60% of patients with carcinomas of the lung, colon or prostate have experienced weight loss prior to beginning chemotherapy. Although the relationship between pretreatment malnutrition (weight loss) and adverse outcome is established, no consistent relationship has been demonstrated between the development of cachexia and tumor size, disease stage, and type or duration of the malignancy. Development of cachexia in the cancer patient is not caused simply by increased energy expenditure by the host or by the tumor. The malignant cachexia is partially related to reduced caloric intake.

Cancer cachexia is not simply a local effect of the tumor. Alterations in protein, fat, and carbohydrate metabolism occur commonly. For example, abnormalities in carbohydrate metabolism include increased rates of total glucose turnover, increased hepatic gluconeogenesis, glucose intolerance and elevated glucose levels. Increased lipolysis, increased free fatty acid and glycerol turnover, hyperlipidemia, and reduced lipoprotein lipase activity are frequently noted. The weight loss associated with cancer cachexia is caused not only by a reduction in body fat stores but also by a reduction in total body protein mass, with extensive skeletal muscle wasting. Increased protein turnover and poorly regulated amino acid oxidation may also be important. Presence of host-derived factors produced in response to the cancer have been implicated as causative agents of cachexia, e.g., tumor necrosis factor-α (TNF) or cachectin, interleukin-1 (IL-1), IL-6, gamma-interferon (IFN), and prostaglandins (PGs) (e.g., PGE_2).

Anorexia, with progressive depletion of body stores leading to the cachectic state, is observed in 50% of cancer-bearing patients. Different mechanisms proposed to explain the pathogenesis of anorexia include: (i) increased production of cytokines such as TNF and IL-1, and (ii) increased serotoninergic activity within the central nervous system

secondary to enhanced availability to the brain of its precursor, tryptophan. Dickerson, J. W. T. et al., 1976, *J. Neurochem* 27: 1245–1247 have suggested that diets should be selected to keep the ratio of plasma tryptophan to the sum of neutral amino acids constant. Cangiano, C., et al., 1994, *Anticancer Res.* 14: 1451–1456 has also disclosed that a close relationship between plasma free tryptophan concentration and anorexia in cancer patients supports the serotoninergic system activity in the pathogenesis of cancer anorexia.

Cancer is characterized primarily by an increase in the number of abnormal cells derived from a given normal tissue, invasion of adjacent tissues by these abnormal cells, and lymphatic or blood-borne spread of malignant cells to regional lymph nodes and to distant metastatic sites. Clinical data and molecular biologic studies indicate that cancer is a multi-step process that begins with minor preneoplastic changes, which may under certain conditions progress to neoplasia causing metabolic effects such as cachexia.

Tumor cells differ from normal cells in their metabolism of fat in that tumor cells consume short-chain and medium-chain fatty acids poorly. For example, tumor-bearing mice fed a diet rich in medium-chain triglycerides had less weight loss with a marked reduction in tumor size compared with animals fed long-chain triglycerides. Moreover, there have been problems reported with the use of high levels of medium-chain triglycerides and use of structured lipids has been suggested in some total parenteral nutrition formulas. Moreover, these structured lipids do not provide the same benefits if administered enterally. U.S. Pat. Nos. 4,906,664 and 5,081,105 disclose the use of certain structured lipids in the treatment of cancer. Preparations for enteral nourishment including varying ratios of ω-6 to ω-3 (2.1:1–3.0:1) have also been used in oncologic patients. However, these preparations used proportionately larger amounts of ω-6 to ω-3 fatty acids. Furthermore, these preparations did not include additional amounts of branched-chain amino acids and antioxidants as set forth in the present invention. The use of the polyunsaturated fatty acid eicosapentaenoic acid is suggested for the treatment of cachexia by inhibiting lipolytic activity of lipolytic agents in body fluids and the activity of the enzyme guanidino-benzoatase. See Tisdale, M. J., and Beck, A., U.S. Pat. No. 5,457,130, issued Oct. 10, 1995; and Tisdale, et al. *Cancer Research* 50: 5022–5026 (August 1990). However, the product taught by Tisdale was in a solid dosage form, requiring an already ill patient to swallow 12–16 capsules per day. This method had serious drawbacks, including difficulty in swallowing, belching, and bad odor.

Thus, the prevention and/or treatment of cachexia and anorexia remain a frustrating problem. Both animal and human studies suggest that nutritional support is largely ineffective in repleting lean body mass in the cancer-bearing host. Randomized trials exploring the usefulness of total parenteral nutrition (TPN) support as an adjunct to cytotoxic antineoplastic therapy have demonstrated little improvement in treatment results. See for example Brennan, M. F., and Burt, M. E., 1981, *Cancer Treatment Reports* 65 (Suppl. 5): 67–68. This, along with a clear demonstration that TPN can stimulate tumor growth in animals suggests the routine use of TPN in cancer treatment is not justified. Kisner, D. L., 1981, *Cancer Treatment Reports* 65 (Suppl. 5): 1–2.

Long chain fatty acid bio-pathways and physiological actions are discussed in U.S. Pat. No. 5,223,285 to DeMichele, et al., the entirely of which is incorporated herein by reference.

Also of interest is U.S. Pat. No. 5,444,054 to Garleb, et al. and a related U.S. Pat. No. 5,780,451 (allowed application

Ser. No. 08/221,349). These documents describe compositions and methods useful in the treatment of ulcerative colitis. Such compositions include a protein source that can be intact or hydrolyzed proteins of high biological value (col. 21); an indigestible oligosaccharide such as fructooligosaccharide; and a lipid blend containing a relatively high proportion of eicosapentaneoic acid, which contributes to a relatively high ω-3 to ω-6 fatty acid ratio.

SUMMARY OF THE INVENTION

The methods of the invention generally comprise inhibiting metabolic and cytokine associated features of cachexia in an individual by administering a nutritional composition comprising an effective amount of ω-3 fatty acids including, but not limited to alpha-linolenic acid, stearidonic acid, eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid, alone or in combination with each other. The invention also relates to administering a nutritional composition comprising effective amounts of branched-chain amino acids, valine, leucine, isoleucine, or mixtures thereof, and with or without a reduced amount of tryptophan and hydroxytryptophan. The invention further provides a method of reducing oxidative damage and anti-cancer drug-induced immunosuppression in a cancer patient by administering a nutritional composition comprising effective amounts of antioxidants including, but not limited to beta-carotene, vitamin C, vitamin E, selenium, or mixtures thereof.

In one aspect, the invention provides a method of preventing the onset of cachexia and/or anorexia, or treating existing cachexia and/or anorexia in a human comprising enterally administering to the human at least:

(a) an oil blend containing ω-6 fatty acids and at least 450 mg of ω-3 fatty acids, the weight ratio of ω-6 fatty acids to ω-3 fatty acids being from about 0.1 to about 3.0; and

(b) a source of amino-nitrogen wherein 15% to 50% by weight of the amino acids of said source of amino-nitrogen are branched-chain amino acids; and

(c) an antioxidant component comprised of at least one nutrient selected from the group comprising beta-carotene, vitamin C, vitamin E, selenium, or mixtures thereof.

These components may be administered in a single composition or in separate vehicles. Preferably, about 15% to about 25% of the amino-nitrogen is provided by branched-chain amino acids; most preferably, about 20%. It is also preferred that the source of amino nitrogen provides tryptophan in an amount of less than about 5.0% by weight of the total amount of the amino acids of said source of amino-nitrogen; more preferably at a level of less than 3% by weight.

In another aspect, the invention provides a method of preventing the onset of anorexia or of treating existing anorexia in a human comprising administering to the human a nutritional composition comprising amino-nitrogen wherein about 5 to 25 grams of branched-chain amino acids selected from valine, leucine, isoleucine, or mixtures thereof are present in an amount from about 15% to about 50% by weight, preferably about 15–25%, of the total amount of amino-nitrogen present in said nutritional composition, and wherein tryptophan in an amount not greater than about 5.0% by weight of the total amount of amino acids is present in said composition and wherein ω-6 and ω-3 fatty acids are present at a weight ratio of from about 0.1 to about 3.0 and at least one antioxidant is present in the nutritional composition.

There is further disclosed a method for preventing immunosuppression in a human comprising administering to the human a liquid nutritional composition comprising:

(a) an oil blend containing ω-6 and ω-3 fatty acids, the weight ratio ω-6 fatty acids to ω-3 fatty acids being about from 0.1 to about 3.0; and

(b) an antioxidant component comprising about 2,500 to about 6,500 micrograms per liter beta-carotene, about 250 to about 1,000 milligrams per liter vitamin C, about 100 to about 500 I.U. per liter vitamin E, and about 75 to about 125 mcg per liter selenium.

There is also disclosed a method of enhancing the transport and efficacy of anticancer drugs in a human having a cancerous condition comprising administering to the human a nutritional composition comprising an oil blend containing ω-6 and ω-3 fatty acids, the weight ratio of total ω-6 fatty acids to ω-3 fatty acids being from about 0.1 to about 3.0.

In another aspect, the invention provides a liquid nutritional composition comprising per liter:

(a) at least 0.45 gm (450 mg) of ω-3 fatty acids and wherein the weight ratio of ω-6 fatty acids to ω-3 fatty acids is from about 0.1 to about 3.0;

(b) at least 50 grams of a source of amino-nitrogen wherein 15 to 50% by weight of the amino-nitrogen is branched-chain amino acids and wherein tryptophan is present in an amount less than about 5.0% by weight of the total amino-nitrogen; and

(c) at least 1 gram of an antioxidant system comprising beta-carotene, vitamin C, vitamin E and selenium.

Generally, such compositions provide much higher levels of the ω-3 fatty acids: preferably from about 1.0 gm to about 100 gm per liter; more preferably, from about 5.0 gm to about 10 gm per liter. Similarly, it is preferred that about 15–25% (typically about 20%) by weight of the source of amino-nitrogen is branched-chain amino acids.

The various methods according to the present invention may be accomplished by feeding a single composition that contains all the components of the invention (ω-6 to ω-3 oil, branched-chain amino acids and antioxidant system) or each component may be fed individually. Further, these methods may be accomplished through the consumption of pills or capsules that contain the elements of the claimed invention. In one embodiment of the invention, a nutritional liquid formulation containing all the elements of the invention is contemplated except for the branched-chain amino acids which may be consumed in the form of a pill or tablet.

In yet another co-embodiment of the invention, a liquid nutritional product contains all the elements of the composition, wherein the branched-chain amino acids are dispersed within the liquid in the form of microcapsules. This administration of the branched-chain amino acids in the form of capsules, tablets, pills and/or microcapsules is advantageous since the organoleptic or taste properties of the amino acids are very objectionable.

In contrast to the prior art, the nutritional composition of the present invention is not restricted to correcting metabolism of just one nutrient class at a time, such as lipids or amino acids. Instead, a preferred nutritional multinutrient composition comprises a balanced formulation containing ω-3 fatty acids, antioxidants, branched-chain amino acids, and with or without a reduced level of tryptophan and 5-hydroxytryptophan. Such a composition can demonstrate strong inhibition of cachexia and anorexia associated with a variety of different cancers (disease states).

In yet another embodiment, the methods further optionally comprise administering the nutritional composition in

combination with cancer chemotherapeutic agents, including but not limited to, 5-fluorouracil, mitomycin-C, adriamycin, chloroethyl nitrosoureas and methotrexate, to improve the transport of the drug into the target cancer cells and ultimately the efficacy of the anticancer agent.

The Examples presented below exemplify the use according to the methods of the invention of ω-3 fatty acids, antioxidants, branched-chain amino acids with or without a reduced level of tryptophan in nutritional therapy of cachexia and anorexia in human patients suffering from different cancers, including, but not limited to, liver, breast, lung, prostate, gastrointestinal and pancreatic cancer.

DETAILED DESCRIPTION OF THE INVENTION

"Cachexia" refers to a state of general ill health and malnutrition. It is often associated with and induced by malignant cancer, and is characterized by loss of appetite, loss of body mass, especially lean body mass, and muscle wasting.

"Anorexia" refers simply to a loss of appetite, whether brought on by medical or psychological factors. Anorexia is often closely associated with, and generally contributes to, the cachexia seen in patients with advanced cancers.

"Fatty acids" refer to a family of carboxylic acids having a hydrocarbon chain, generally from about 12 to 24 carbons long. When unsaturated (having a double bond) at least one point in the hydrocarbon chain, such fatty acids are designated by the position of the first double bond. ω-3 fatty acids have a first double bond at the third carbon from the methyl end of the chain; and include, but are not limited to, α-linolenic acid, stearidonic acid, eicosapentaenoic acid ("EPA"), docosapentaenoic acid and docosahexaenoic acid ("DHA") and the like. ω-6 fatty acids have a first double bond at the sixth carbon from the methyl end of the chain; and include, but are not limited to, linoleic acid, γ-linolenic acid, arachidonic acid ("AA"), and the like. The ratio of ω-6 fatty acids to ω-3 fatty acids is simply the ratio of the total amounts (usually expressed as weight) of each type.

Branched-chain amino acids are amino acids that have a fork or branch in the side chain. These include primarily those having a carbon-carbon branch, i.e. valine, leucine and isoleucine; but may also include other types of branches.

"Nutritional matrix" as used herein refers to a delivery vehicle that contains fats, amino nitrogen and carbohydrates and provides some or all of the nutritional support for a patient in the recommended daily amounts. Frequently a nutritional matrix will contain vitamins, minerals, trace minerals and the like to provide balanced nutrition.

"Cytokines" as used herein refer to the causative agents of cachexia in the cancer patient, produced by the individual in response to the presence of cancer, and include, but are not limited to, tumor necrosis factor (TNF) or cachectin, interleukin-1 (IL-1), IL-6 and gamma-interferon (IFN). TNF is produced by the macrophages in response to nonspecific stimuli including cancer, infection, trauma and stress. The mechanism of action in cancer cachexia involves an immune response to the tumor with the production of cytokines, which not only mediate tumor lysis but also the metabolic changes seen in cancer cachexia through specific TNF receptors and/or via the induction of other cytokine receptors.

While not intending the invention to be limited to any particular theory of operation, applicants describe below a probable mechanism. A mode of action of cytokines is mediated via interactions with receptors on the plasma membrane. This is typically defined as a "signal transduction event." In general, a cytokine receptor consists of an extracellular domain, a transmembrane region spanning the phospholipid bilayer of the plasma membrane, and an intracellular domain having either enzymatic activity or binding other molecules, so that a signal is delivered inside the cell in response to the cytokine ligand interaction. The signal transduction mechanisms involve second messengers, including phospholipases, adenylate cyclase and cyclic AMP, inositol phosphates, diacylglycerols and protein kinase C. More particularly, phospholipase A2 generates arachidonic acid, a precursor of dienoic prostaglandins, thromboxanes, prostacyclin and leukotrienes of the 4 series.

Cytokines such as TNF and IL-1 stimulate production of arachidonic acid metabolites which are important to their inflammatory and tissue damaging actions and are responsible for immunosuppression in general, and in exacerbating some paraneoplastic conditions including metabolic changes seen in cancer cachexia.

The invention is based, in part, on a method of inhibiting signal transduction and cytokine activity using nutritional compositions comprising high levels of ω-3 fatty acids, in particular, of the long chain (e.g. 20 or more carbons) ω-3 fatty acids, eicosapentaenoic (EPA) and docosahexaenoic (DHA).

Since administration of EPA and DHA results in a reduction of arachidonic acid in membrane phospholipids, such an effect not only diminishes the supply of arachidonic acid as a precursor for the dienoic eicosanoids but also inhibits their production through competitive inhibition by EPA. The cyclooxygenase and lipoxygenase metabolites of EPA have attenuated activity. Furthermore, ω-3 fatty acids, alpha-linolenic and stearidonic can be converted through elongation/desaturation to EPA; and similarly, DHA can be retroconverted to EPA. Thus, the methods of invention comprise methods of inhibiting cytokine activity (e.g., TNF, IL-1) and cancer cachexia by interfering with signal transduction at the receptor level and inhibiting arachidonic acid metabolism.

Incorporation of ω-3 fatty acids in membrane phospholipids not only alters the activity of membrane-associated enzymes (e.g., phospholipase A2) but also alters the balance between constituent saturated and unsaturated fatty acids and regulation of membrane fluidity, facilitates the transport of anticancer drugs into the cancer cells and thus enhances the efficacy of the drugs. Alberts, A. W., et al., 1978, *Biochim. Biophys. Acta* 509:239–250. In addition, the inhibition of arachidonic acid metabolism results in prevention and/or reversal of immunosuppression by reducing the production of prostaglandins and leukotrienes (PGE2 and LTB4), which are immunosuppressive.

The invention also provides a method of reducing the concentration of brain tryptophan and serotonin to prevent or inhibit premature satiety and cancer cachexia and/or anorexia in a cancer patient in whom the prevention and treatment of cancer anorexia is desired by administering effective amounts of branched-chain amino acids, valine, leucine, isoleucine, or mixtures thereof, and with or without a reduced amount of tryptophan.

The methods and compositions of the invention provide a method of manipulating the concentration of brain tryptophan by: (i) increasing the branched-chain amino acids, which provide competition for tryptophan for penetration across the blood-brain barrier; and (ii) reduced levels of tryptophan and 5-hydroxytryptophan in relation to branched-chain amino acids in the nutritional composition

of the invention. Such an intervention can increase appetite and thus prevent and/or treat cancer anorexia.

The methods and compositions of the invention also provide a method of reducing the risk or progression of certain symptoms of cancer, such as cancer cachexia and anorexia by administering antioxidant nutrients including, but not limited to, beta-carotene, vitamin C, vitamin E, selenium, or mixtures thereof. Epidemiological evidence indicates that a combination of beta-carotene, vitamin E and selenium can effect a reduction in cancer risk in some populations. Blot, N. J. et al., 1993, *J. Natl. Cancer Inst.* 85: 1483–1492. Furthermore, vitamin E is added to satisfy any additional requirements as a result of a higher intake of ω-3 polyunsaturated fatty acids. By the administration of the antioxidant nutrients of the invention to cancer patients having cachexia and whose immune system has been depressed on account of chemotherapy and/or oxidative burden, improvements in the nutritional status, as well as prevention and treatment of immunosuppression and cachexia can be achieved.

Nutritional support in the cancer patient can be categorized as (i) supportive, in which nutrition support is instituted to prevent nutrition deterioration in the adequately nourished patient or to rehabilitate the depleted patient before definitive therapy; (ii) adjunctive, in which nutrition support plays an integral role in the therapeutic plan; and (iii) definitive, in which aggressive nutrition support is required for the patient's existence. The routes for providing nutrition support include an oral diet, tube feeding and peripheral or total parenteral nutrition. The preferred embodiment for nutritional methods and compositions of the invention is by the oral route.

An alternate to oral feeding is tube feeding by means of nasogastric, nasoduodenal, esophagostomy, gastrostomy, or jejunostomy tubes.

A typical nutritional composition useful in the method of this invention will have a caloric distribution as follows: about 12 to 24% (target 21%) from a source of amino-nitrogen, about 40 to 65% (target 61%) from carbohydrate and about 10 to 35% (target 18%) from fat. More particularly, the oil blend may comprise approximately 30% of ω-3 fatty acids, preferably largely consisting of eicosapentaenoic acid and docosahexaenoic acid. Dietary oils used in the preparation of the nutritional composition generally contain ω-3 fatty acids in the triglyceride form and include, but are not limited to canola, medium chain triglycerides, fish, soybean, soy lecithin, corn, safflower, sunflower, high-oleic sunflower, high-oleic safflower, olive, borage, black currant, evening primrose and flaxseed oil. Table 1 sets forth both preferred amounts and ranges for an oil blend useful in the invention. Specifically, the weight ratio of ω-6 fatty acids to ω-3 fatty acids in the lipid blend according to the invention is about 0.1 to 3.0. The daily delivery of ω-3 fatty acids should be at least 450 mg and may vary depending on body weight, sex, age and medical condition of the individual. As mentioned, higher levels are desired for adult human consumption: for example, from about 0.5 to 50 gm daily, more preferably from about 2.5 to 5 gm daily.

TABLE 1

OIL BLEND (% total weight of lipid blend)

OIL	PREFERRED	RANGE
Canola	9.3%	5.0–40.0%
MCT	16.2%	10.0–50.0%
Fish	65.0%	25.0–80.0%
Soybean	5.5%	3.0–30.0%
Soy lecithin	4.0%	2.0–6.0%

Table 2 presents the fatty acid profile of an exemplary oil blend useful in the present invention. The weight ratio of the total ω-6 fatty acids to the total ω-3 fatty acids in this embodiment is 0.26 to 1 which is within the claimed range for this invention.

TABLE 2

FATTY ACID PROFILE (% of total fatty acids by weight)

OIL	%
Caproic (6:0)	0.53
Capyrlic (8:0)	10.35
Capric (10:0)	7.16
Lauric (12:0)	0.29
Myristic (14:0)	3.53
Palmitic (16:0)	7.41
Palmitoleic (16:1ω7)	5.73
Stearic (18:0)	1.39
Oleic (18:1ω9)	15.23
Linoleic (18:2ω6)	7.21
Gamma-linoleic (18:3ω6)	0.21
Alpha-linoleic (18:3ω3)	2.21
Stearidonic (18:4ω3)	2.40
Arachidic (20:0)	0.13
Eicosenoic (20:1ω9)	0.74
Arachidonic (20:4ω6)	0.87
Eicosapentaenoic (20:5ω3)	17.14
Erucic (22:1ω9)	0.17
Docosapentaenoic (22:5ω3)	2.08
Docosahexaenoic (22:6ω3)	7.73
Nervonic (24:1ω9)	0.14
Others	7.35
Total	100.00

TABLE 3:

LIPID BLEND CHARACTERISTICS	
% ω-3 fatty acids	30.51
% ω-6 fatty acids	9.67
% ω-9 fatty acids	15.28
% saturated fatty acids	27.07
% monounsaturated fatty acids	19.33
% polyunsaturated fatty acids	40.17
ω-6/ω-3 ratio	0.32
18:2ω6/18:3ω3 ratio	4.26
18:3ω3, % total energy	0.33
18:2ω6, % total energy	1.41
18:1ω9, % total energy	2.42
PUFAs, % total calories	7.23
saturated fatty acids, % total calories	4.87
EPA (20:5ω3) per 8 oz container, g	1.09
DHA (22:6ω3) per 8 oz container, g	0.46

Table 3 (above) sets forth selected characteristics of an oil blend useful in the method of this invention. However, it will be realized that the characteristics may vary among other formulas useful for this invention, depending on the specific oils added and the ratios in which they are used.

An amino acid profile for a nutritional composition useful in the invention is presented in Table 4.

TABLE 4

AMINO ACID PROFILE

Amino Acid	g/100 g Protein
Aspartic Acid	7.08
Threonine	4.34
Serine	5.68
Glutamic Acid	20.58
Proline	10.55
Glycine	1.81
Alanine	3.04
Valine	5.90
Methionine	2.78
Isoleucine	4.77
Leucine	9.08
Tyrosine	4.79
Phenylalanine	4.96
Histidine	2.67
Lysine	7.27
Arginine	3.15
Tryptophan	0.99
Cystine	0.56
Total BCAA	19.75

The total amount of branched-chain amino acids ("BCAA") useful in the present invention is about 15–50 g/100 g protein (i.e. percent), preferably about 15–25 g/100 g. Thus, an 8 oz container of the nutritional composition would contain up to about 8g BCAAs per 16 grams of total protein. The daily delivery of BCAAs is about 5–26 g. In order to deliver such a high amount of the BCMs, and because the BCMs impart an unpleasant taste, the nutritional composition may be accompanied by 1–3 gelatin capsules containing BCAAs to provide the additional amount required above the inherent amount present in the liquid product. The preferred BCAAs are, but are not limited to, leucine, isoleucine and valine, and are predominantly bitter in taste. Therefore, administering the additional BCAAs in encapsulated form avoids taste problems which are encountered with the use of quantities greater than 20 g/100 g protein of BCAAs in the liquid product. The microencapsulated BCAAs may also be mixed with taste masking compounds including, but not limited to, polyphosphates, cyclodextrin (a cyclic glucose oligomer) and Thaumatin (a proteinaceous intense sweetener).

A representative antioxidant profile useful in the method of the invention is presented in Table 5 with range values and a preferred embodiment.

TABLE 5

ANTIOXIDANT PROFILE

Antioxidant	Preferred	Range
Beta-carotene	5,000 μg/L	2,500–6,500 μg/L
Vitamin E	300 IU/L	100–500 IU/L
Vitamin C	650 mg/L	250–1,000 mg/L
Selenium	90 μg/L	78.8–125 μg/L

The overall nutrient profile of this example is set forth in Table 6. In a specific embodiment of this invention, the nutritional product provides at least 100% of the U.S. RDA for vitamins and minerals in 1184 mL (five 8 fluid ounce servings), which would provide 1184 kcal per day.

If used as a sole source of nutrition, and assuming a 2000 kcal diet, between 8 and 9 servings (237 mL; 8 fluid ounces) of this illustrative formulation would be required. However, as seen from example IV below, there is benefit derived from supplementation with as few as two servings per day. Thus a minimum daily amount of long chain ω-3 fatty acids is preferably about 3 grams, calculated as (1.06 g EPA+0.46 g DHA) times 2 8 oz servings. Of course, if more servings are consumed to provide additional calories, more ω-3 fatty acids will be administered, up to a practical maximum of about 14 grams per day (about 9 servings at same fatty acid levels). Levels of the fatty acids, antioxidants and/or source of amino nitrogen on a per liter basis are not crucial, except to the extent that a reasonable volume of fluid should supply the recommended daily amounts consistent with the invention. Determination of a reasonable volume is easily within the ambit of those skilled in the art, especially in view of the specific guidance found in the examples.

TABLE 6

NUTRIENT PROFILE

Nutrient	Qty/Liter
Protein, g	67.40
Fat, g	27.20
Carbohydrate, g	207.00
Total Dietary Fiber, g	10.70
Indigestible Oligosaccharide (FOS), g	12.40
Gum Arabic, g	9.10
Soy Polysaccharide, g	1.60
Beta-carotene, μg	5000
Vitamin A, IU	5500
Vitamin D, IU	800.00
*Vitamin E, IU	300.00
Vitamin K, μg	135.00
Vitamin C, mg	650.00
Folic Acid μg	1900
Thiamine, mg	6.50
Riboflavin, mg	5.00
Vitamin B_6, mg	5.00
Vitamin B_{12}, μg	18.00
Niacin, mg	40.00
Choline, mg	525.00
Biotin, μg	750.00
Pantothenic Acid, mg	24.00
Sodium, mg	1500
Potassium, mg	2000
Chloride, mg	1519
Calcium, mg	1800
Phosphorous, mg	1250
Magnesium, mg	450.00
Iodine, μg	175.00
Copper, mg	2.61
Zinc, mg	29.20
Iron, mg	22.20
Selenium, μg	90.00
Chromium, μg	125.00
Molybdenum, μg	206.00
Carnitine, mg	150.00
Taurine, mg	275.00
Kcal/mL	1

*d-alpha-tocopherol (all natural form) or dl-alpha-tocopherol acetate, or a combination of the two.

The following specific examples are set forth to illustrate various preferred embodiments of the invention but the scope of the invention is defined by the appended claims.

EXAMPLE I

The specific list of materials for manufacturing the nutritional cancer product of this Example I is presented in Table 7. Of course, various changes in specific ingredients and quantities may be made without departing from the scope of the invention.

TABLE 7

LIST OF MATERIALS

INGREDIENT	AMOUNT
WATER	31,605.21 kg
GUM ARABIC	437.84 kg
ULTRATRACE/TRACE MINERAL PREMIX	14.50 kg
ZINC SULFATE	2969.89 gm
FERROUS SULFATE	2856.50 gm
MANGANESE SULFATE	784.60 gm
CUPRIC SULFATE	423.11 gm
SODIUM MOLYBDATE	21.39 gm
CHROMIUM CHLORIDE	20.80 gm
SODIUM SELENITE	8.11 gm
CITRIC ACID	894.94 gm
SUCROSE (Carrier)	6520.67 gm
POTASSIUM CITRATE	50.00 kg
SODIUM CITRATE	95.00 kg
POTASSIUM IODIDE	9.00 gm
POTASSIUM CHLORIDE	91.00 kg
CORN SYRUP SOLIDS	5630.96 kg
MALTODEXTRIN	1407.52 kg
MAGNESIUM PHOSPHATE DIBASIC	131.00 kg
CALCIUM PHOSPHATE TRIBASIC (PREFERABLY MICRONIZED)	47.50 kg
CALCIUM CARBONATE	122.50 kg
SUGAR (SUCROSE)	852.77 kg
FRUCTOOLIGOSACCHARIDE	509.96 kg
MEDIUM CHAIN TRIGLYCERIDES (FRACTIONATED COCONUT OIL)	172.69 kg
CANOLA OIL	99.13 kg
SOY OIL	58.63 kg
57% VITAMIN A PALMITATE	250.00 gm
2.5% VITAMIN D	35.00 gm
D-ALPHA-TOCOPHEROL ACETATE (R,R,R)	10.65 kg
PHYLLOQUINONE	6.50 gm
30% BETA-CAROTENE	824.00 gm
SOY LECITHIN	42.64 kg
SODIUM CASEINATE	1427.04 kg
PARTIALLY HYDROLYZED SODIUM CASEINATE	1427.04 kg
SOY POLYSACCHARIDE	85.28 kg
75% WHEY PROTEIN CONCENTRATE	184.46 kg
REFINED DEODORIZED SARDINE OIL	692.87 kg
ASCORBIC ACID	37.08 kg
45% POTASSIUM HYDROXIDE	25.96 kg
TAURINE	12.00 kg
WATER SOLUBLE VITAMIN PREMIX	4.50 kg
NIACINAMIDE	1688.60 gm
CALCIUM PANTOTHENATE	1092.24 gm
THIAMINE CHLORIDE HYDROCHLORIDE	278.78 gm
PYRIDOXINE HYDROCHLORIDE	268.34 gm
RIBOFLAVIN	217.87 gm
FOLIC ACID	37.82 gm
BIOTIN	32.87 gm
CYANOCOBALAMIN	0.75 gm
DEXTROSE (Carrier)	882.74 gm
FOLIC ACID	43.50 gm
CHOLINE CHLORIDE	25.00 kg
L-CARNITINE	7.00 kg
ARTIFICIAL STRAWBERRY FLAVOR	31.75 kg
ARTIFICIAL CREAM FLAVOR	18.14 kg
FD & C Red Dye No. 3	1,220.16 gm

The liquid nutritional product of the present invention was manufactured by preparing three slurries which are blended together, combined with refined deodorized sardine oil, heat treated, standardized, packaged and sterilized. The process for manufacturing 45,360 kg (100,000 pounds) of the liquid nutritional product, using the List of Materials from Table 7, is described in detail below.

A carbohydrate/mineral slurry is prepared by first heating about 6,260 kg of water to a temperature in the range of about 71° C. to 77° C. with agitation. The gum arabic is then added to the water using a mixing apparatus. Next the ultratrace/trace mineral premix is added to the water and dissolved by agitating the resultant solution for at least one minute. The following minerals are then added, in the order listed, with high agitation: potassium citrate, sodium citrate, potassium iodide and potassium chloride. The corn syrup solids (Grain Processing Corporation, Muscatine, Iowa, U.S.A. under the trade designation "Maltrin M-200") and maltodextrin (Grain Processing Corporation, trade designation "Maltrin M-100") are then added to the slurry and the temperature of the slurry is maintained at about 71° C. with high agitation for at least about 20 minutes.

Add magnesium phosphate dibasic, calcium phosphate tribasic, and calcium carbonate to the slurry. Sugar (sucrose), and fructooligosaccharide (Golden Technologies Company, Golden, Colo., U.S.A. under the trade designation "Nutriflora-P- Fructo-oligosaccharide Powder (96%)") are added to the slurry. The completed carbohydrate/mineral slurry is held with high agitation at a temperature in the range of about 60–66° C. for not longer than 12 hours until it is blended with the other slurries.

An oil slurry is prepared by combining and heating the medium chain triglycerides (fractionated coconut oil), canola oil and soy oil to a temperature in the range of about 32–43° C. with agitation. The 57% vitamin A palmitate, 2.5% vitamin D_3, D-alpha-tocopherol acetate (R,R,R form; Distillation Products Industries, a division of Eastman Kodak Chemical Company, Rochester, N.Y. U.S.A. under the trade designation "Eastman Vitamin E 6-81 D-Alpha Tocopherol Acetate Concentrate"), phylloquinone and 30% beta-carotene are added to the slurry with agitation. The soy lecithin is then added to the slurry with agitation. The completed oil slurry is held under moderate agitation at a temperature in the range of about 32–43° C. for not longer than 12 hours until it is blended with the other slurries.

A protein-and-fiber-in-water slurry is prepared by first heating about 19,678 kg of water to a temperature in the range of about 60–63° C. with agitation. Sodium caseinate, partially hydrolyzed sodium caseinate (distributed by New Zealand Milk Products, Santa Rosa, Calif., U.S.A. under the trade name Alanate 167) and soy polysaccharide are blended into the slurry using a mixing apparatus. The temperature of the slurry is lowered to about 57–60° C. and then the 75% whey protein concentrate is added to the slurry using a mixing apparatus. The completed protein-and-fiber-in-water slurry is held under agitation at a temperature in the range of about 54–60° C. for not longer than 2 hours before being blended with the other slurries.

The oil slurry and the protein-and-fiber-in-water slurry are blended together with agitation and the resultant blended slurry is maintained at a temperature in the range of about 54–66° C. After waiting for at least one minute the carbohydrate/mineral slurry is added to the blended slurry from the preceding step with agitation and the resultant blended slurry is maintained at a temperature in the range of about 54–66° C. The vessel which contained the carbohydrate/mineral slurry should be rinsed with about 220 kg of water and the rinse water should be added to the blended slurry. The refined deodorized sardine oil (distributed by Mochida International Company, Limited, Shinjuku-ku, Tokyo, Japan under the trade designation "50% Omega-3 marine oil EPA:DHA 28:12 with 0.8% mixed tocopherol as antioxidant") is then added to the slurry with agitation. (In a most preferred method of manufacture the sardine oil would be slowly metered into the product as the blend passes through a conduit at a constant rate.) Preferably after at least 5 minutes the pH of the blended slurry is determined. If the pH of the blended slurry is below 6.55, it is adjusted with dilute potassium hydroxide to a pH of 6.55 to 6.8.

After waiting a period of not less than one minute nor greater than two 45 hours the blended slurry is subjected to deaeration, Ultra-High-Temperature (UHT) treatment, and homogenization, as described below:

A. Use a positive pump for supplying the blended slurry for this procedure.

B. Heat the blended slurry to a temperature in the range of about 66–71° C.

C. Deaerate the blended slurry to 25.4–38.1 cm of Hg.

D. Emulsify the blended slurry at 61–75 Atmospheres.

E. Heat the blended slurry to a temperature in the range of about 120–122° C. by passing it through a plate/coil heat exchanger with a hold time of approximately 10 seconds.

F. UHT heat the blended slurry to a temperature in the range of about 144–147° C. with a hold time of approximately 5 seconds.

G. Reduce the temperature of the blended slurry to be in the range of about 120–122° C. by passing it through a flash cooler.

H. Reduce the temperature of the blended slurry to be in the range of about 71–82° C. by passing it through a plate/coil heat exchanger.

I. Homogenize the blended slurry at about 265 to 266 Atmospheres.

J. Pass the blended slurry through a hold tube for at least 16 seconds at a temperature in the range of about 74–85° C.

K. Cool the blended slurry to a temperature in the range of about 1–70° C. by passing it through a large heat exchanger.

Store the blended slurry at a temperature in the range of about 1–7° C., preferably with agitation.

Preferably at this time appropriate analytical testing for quality control is conducted. Based on the test results an appropriate amount of dilution water (10–38° C.) is added to the blended slurry with agitation.

A vitamin solution, a flavor and a color solution are prepared separately and then added to the blended slurry.

The vitamin solution is prepared by heating about 394 kg of water to a temperature in the range of about 43–66° C. with agitation, and thereafter adding the following ingredients, in the order listed: Ascorbic Acid, 45% Potassium Hydroxide, Taurine, Water Soluble Vitamin Premix, Folic Acid, Choline Chloride, and L-Carnitine. The vitamin solution is then added to the blended slurry with agitation.

The flavor solution is prepared by adding the artificial strawberry flavor and artificial cream flavor to about 794 kg of water with agitation. A nutritional product according to the present invention has been manufactured using an artificial strawberry flavor distributed by Firmenich Inc., Princeton, N.J., U.S.A. under the trade designation “Art. strawberry 57.883/A” and an artificial cream flavor distributed by Firmenich Inc. under the trade designation “Art Cream 59.200/A”. The flavor solution is then added to the blended slurry with agitation.

A color solution is prepared by adding the FD&C Red Dye No. 3 to about 121 kg of water with agitation. The color solution is then added to the blended slurry with agitation.

If necessary, diluted potassium hydroxide is added to the blended slurry such that the product will have a pH in the range of 6.4 to 7.0 after sterilization. The completed product is then placed in suitable containers and subjected to sterilization. Of course, if desired aseptic processing could be employed.

The product made according to the procedure of this example contains the oil blend of Table 8, below, the the fatty acid properties of Tables 2 and 3, and the amino acid profile of Table 4, all set forth above.

TABLE 8

OIL BLEND
(% total weight of lipid blend)

OIL	Percent Total Lipids
Canola	9.3%
MCT	16.2%
Fish	65.0%
Soybean	5.5%
Soy lecithin	4.0%

EXAMPLE II

The objective of this experiment was to evaluate the organoleptic characteristics of nutritional composition of the invention fortified by the addition of branched-chain amino acids incorporated at two different levels. To measure organoleptic properties, three taste standards, described in Table 9, were prepared to rank the bitter and sour intensity of the test compositions containing branched-chain amino acids.

TABLE 9:

TASTE INTENSITY SCALE

Standard	Basic Taste	Intensity	Concentration* by Weight	Representative Products
1	Sour	1	0.05% Citric Acid	Milk Chocolate, Coffee Whole Peanuts
2	Bitter	1	0.05% Caffeine	Milk Chocolate
3	Bitter	2	0.10% Caffeine	Beer

*Aqueous solutions

Two test compositions (designated “high” and “low”) were prepared by adding selected branched-chain amino acids (“BCAA”) to the liquid nutritional composition of Example I. A control composition of Example I that did not contain the supplemental branched-chain amino acids was also evaluated for flavor characteristics. The specific amino acids and amounts added are given in Table 10. In both test compositions the branched-chain amino acids did not completely disperse in the nutritional composition due to their hydrophobic nature, and small clumps of branched-chain amino acids were visible in the matrix. The test compositions were evaluated and the results of the organoleptic test scoring are also set forth in Table 10.

TABLE 10

BRANCHED-CHAIN AMINO ACID FORTIFICATION

	“high” test	“low” test	control
BCM in gm/237 mL serving			
valine	2.5	1.3	0
leucine	2.5	1.3	0
isoleucine	2.5	1.3	0
Total	7.5	3.9	0

TABLE 10-continued

BRANCHED-CHAIN AMINO ACID FORTIFICATION	"high" test	"low" test	control
TASTE TEST SCORE			
bitter	1.5 to 2	1.5	none
sour	0.5	0.5	none

Based on the results of this taste session, the evaluators collectively agreed that the bitter and sour flavor notes attributed to the branched-chain amino acids are less than ideal for a ready-to-use oral nutritional composition. Thus, in one embodiment of this invention, any additional branched-chain amino acids are supplied to patient in the form of a pill or capsule distinct from the liquid nutritional of the invention.

EXAMPLE III

The effect of nutritional intervention with ω-3 fatty acids, branched-chain amino acids and antioxidants in the nutritional compositions of the invention, on prevention and treatment of cachexia can be monitored by any of the methods known to one skilled in the art, including but not limited to measuring: (i) food intake, body weight and anthropometric measurements; (ii) serum levels of lipids, fatty acids, amino acids and antioxidants; (iii) levels of serologic markers where appropriate, e.g., carcinoembryonic (CEA) antigens, serotonin, C-reactive protein, TNF and IL-1; (iv) changes in the morphology of tumors using techniques such as computed tomographic (CT) scan, ultrasonography, magnetic resonance imaging (MRI) and position emission tomography (PET).

Patients with hepatocellular carcinoma showing symptoms of cachexia are provided with the nutritional product of the invention with small, frequent feedings after surgical resection if the liver tumor is localized and small, or along with a regimen of chemotherapy. The liver functions and characteristics of the hepatic carcinomas are tested by procedures known in the art.

The daily nutritional management of liver cancer, therefore, includes administration of 2 to 4 containers of 8 ounce servings (237 mL) of the nutritional composition providing a daily amount of: (i) combined EPA and DHA in the range of 3 to 6 g, with the preferred dosage being about 3 g; (ii) branched-chain amino acids in the range of 5 to 25 g, with the preferred dosage being about 10–15 g branched-chain amino acids; and (iii) vitamin C in the range of 125 to 500 mg, with the preferred dosage being about 300 mg vitamin C; (iv) vitamin E in the range of 50 to 250 IU, with the preferred dosage being about 150 IU vitamin E; (v) beta-carotene in the range of 1250 to 3250 μg, with the preferred dosage being about 2500 beta-carotene μg; (vi) selenium in the range of 40 to 60 μg, with the preferred dosage being about 45 μg selenium. The effect of nutritional intervention on cancer cachexia and anorexia are monitored at monthly intervals (or as recommended in the clinical follow-up) as known in the art, and depending on the results obtained, the therapeutic regimen is developed to maintain and/or boost the weight gain by the patient, with the ultimate goal of achieving tumor regression and complete eradication of cancer cells.

For the underweight breast cancer patient on adjuvant chemotherapy, administration of the nutritional composition of the invention is started any time after surgery. The nutritional composition used in breast cancer patients is designed to maintain an adequate intake in spite of nausea, mucositis, and other side effects of chemotherapy. Patients receiving radiation therapy for breast cancer receive effective amounts of the nutritional composition to promote maintenance and repair of body tissue. The therapeutic and/or prophylactic regimens used in breast cancer patients are the same as those described in Section 6 above for patients recovering from hepatocellular carcinoma. The procedures of monitoring the patient under clinical evaluation for prevention and treatment of cachexia and anorexia in breast cancer are known in the art.

EXAMPLE IV

A pilot study was conducted to assess the effectiveness of a specific formula in ameliorating the cachexia of cancer patients. The formula of Example I was prepared. In addition to other nutrients, it contained (per two 237 mL servings) the long-chain ω-3 fatty acids, the fructooligosaccharide ("FOS") and the antioxidant system specified in Table 10.

TABLE 10

TRIAL PRODUCT

Ingredient	Amount per 2 × 237 mL servings
EPA ω3	2.0 gm
DHA ω-3	0.92 gm
fructooligosaccharide	5.8 gm
beta carotene	2.8 mg
vitamin G	300. mg
vitamin E	150. IU
selenium	58. mcg

In the pilot clinical trial of this example, ten patients with pancreatic cancer were evaluated. These patients were cachectic and losing weight at a mean rate of 0.86 kg per week over an average of 22 weeks (range: 11 to 56 weeks) prior to the trial. Over a three week trial period, patients consumed an average of two 237 mL (8 fluid ounces) servings per day as a supplement to their diets. After the trial period the group demonstrated a mean increase in weight of 2.1 kg (up from baseline), which translates to a mean weekly weight gain of 0.7 kg (See Table 11).

TABLE 11

Patients' Age, Gender and Weight Status

Patient	Patient Age and Gender	Mean Weekly Wt (kg) Change up to Baseline	Base-line weight (kg)	Weight after three week trial (kg)	Mean Weekly Wt change during trial
1	56f	−0.4	51	51.75	0.25
2	64m	−1.2	67	na	na
3	70m	−0.5	61	67.5	2.17
4	60m	−0.9	43	44	0.33
5	53f	−1.4	90	90.5	0.17
6	51f	−1.2	44.5	45.5	0.33
7	67m	−0.6	57	58	0.33
8	75f	−0.8	55	59	1.33
9	57m	−0.6	69	na	na
10	53f	−1	57.5	na	na
Mean		−0.86			0.7

In addition, much of the weight gained was lean body mass. The group demonstrated a mean increase in lean body mass of 2.1% and a decrease in C-reactive protein ("CRP") levels (See Table 12). Serum CRP is a biochemical surrogate for the presence and progress of cancer cachexia, and shows

a strong positive correlation. (Falconer, J. S. et al., *Cancer* 1995, 75:2077). Patients with serum CRP levels >10 mg/L are frankly cachectic. The mean CRP level at baseline was 31.5 and this dropped to about 10 after 3 weeks on the experimental formula of the invention. Thus, the invention improves cachexia in pancreatic cancer patients.

TABLE 12

Patients' Lean Body Mass and CRP

Patient	% Lean Body Mass at Baseline	% Lean Body Mass after trial	Change in % Lean Body Mass	Baseline CRP mg/L	CRP after 3 week trial mg/L
1	79.4	82.9	3.5	69	<10
2	85.6	na	na	81	na
3	84	92.2	8.2	27	<10
4	85.6	86.3	0.7	<10	<10
5	73.7	75	1.3	<10	<10
6	79.7	80.7	1	63	<10
7	90.2	91.9	1.7	<10	10
8	81.4	80.1	−1.3	<10	<10
9	82.6	na	na	25	na
10	86.9	na	na	<10	na
Mean	82.9	84.2	2.1	31.5	10*

*CRP values read as <10 are assumed to be 10 for calculation of mean

The present invention is not to be limited to the scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications and patents are cited herein, the disclosures of which are incorporated by reference in their entireties.

We claim:

1. A liquid nutritional composition comprising:

(a) at least 1000 mg per liter of ω-3 fatty acids, wherein the weight ratio of ω-6 fatty acids to ω-3 fatty acids is from about 0.1 to about 1.0;

(b) at least 50 grams per liter of a source of amino-nitrogen, wherein 15 to 50% by weight of the amino-nitrogen is branched-chain amino acids, and wherein tryptophan is present in an amount less than about 5.0% by weight of the total amino-nitrogen, and;

(c) at least 1 gram per liter of an antioxidant system comprising beta-carotene, vitamin C, vitamin E and selenium.

2. The composition according to claim **1** in which said ω-3 fatty acids are present in a quantity of about 1.0 gram to about 100 grams per liter.

3. The composition according to claim **1** in which said ω-3 fatty acids are present in a quantity of about 5.0 grams to about 100 grams per liter.

4. The composition according to claim **1** in which said ω-3 fatty acids are present in a quantity of about 5.0 grams to about 10.0 grams per liter.

5. The composition according to any one of claims **2**–**4** in which 15% to 25% by weight of the amino-nitrogen is branched-chain amino acids.

6. The composition claim **5** in which said tryptophan is present in an amount of less than 3.0% by weight a nitrogen.

7. A liquid nutritional composition comprising:

a) at least 1000 mg per liter of ω-3 fatty acids, wherein the weight ratio of ω-6 fatty acids to ω-3 fatty acids is about 0.1 to about 1.0;

(b) at least 50 grams per liter of a source of amino-nitrogen, wherein 15 to 50% by weight of the amino-nitrogen is branched-chain amino acids, and wherein tryptophan is present in an amount less than about 5.0% by weight of the total amino-nitrogen, and;

c) an antioxidant component comprising about 2,500 to about 6,500 micrograms per liter of beta-carotene, about 250 to about 1,000 milligrams per liter of vitamin C, about 100 to about 500 I.U. per liter of vitamin E, and about 75 to about 125 micrograms per liter of selenium.

8. The composition according to claim **7** in which said ω-3 fatty acids are present in a quantity of about 1.0 gm to about 100 grams per liter.

9. The composition according to claim **7** in which said ω-3 fatty acids are present in a quantity of about 5.0 grams to about 100 grams per liter.

10. The composition according to claim **7** in which said ω-3 fatty acids are present in a quantity of about 5.0 grams to about 10.0 grams per liter.

11. The composition according to any one of claims **7**–**10** in which 15% to 25% by weight of the amino-nitrogen is branched-chain amino acids.

12. The composition according to claim **11** in which said tryptophan is present in an amount of less than 3.0% by weight of the total amino-nitrogen.

13. A liquid nutritional composition comprising:

a) optionally at least 50 grams per liter of a source of amino-nitrogen, wherein 15 to 50% by weight of the amino-nitrogen is branched-chain amino acids, and wherein tryptophan is present in an amount less than about 5.0% by weight of the total amino-nitrogen;

b) an antioxidant component comprising about 2,500 to about 6,500 micrograms per liter of beta-carotene, about 250 to about 1,000 milligrams per liter of vitamin C, about 100 to about 500 I.U. per liter of vitamin E, and about 75 to about 125 microgram per liter of selenium, and;

c) an oil blend further comprising 5–40 wt. % canola oil, 10–50 wt. % medium chain triglyceride oil, 25–80 wt. % fish oil, 3–30 wt. % soybean oil, and 2–6 wt. % soy lecithin.

14. The liquid nutritional according to claim **13** in which said oil blend further comprises 9.3 wt. % canola oil, 16.2 wt. % medium chain triglyceride oil, 65 wt. % fish oil, 5.5 wt. % soybean oil, and 4.0 wt. % soy lecithin.

15. A liquid nutritional composition comprising:

a) an antioxidant component comprising about 2,500 to about 6,500 micrograms per liter of beta-carotene, about 250 to about 1,000 milligrams per liter of vitamin C, about 100 to about 500 I.U. per liter of vitamin E, and about 75 to about 125 microgram per liter of selenium, and;

b) at least 1000 mg per liter of ω-3 fatty acids, wherein the weight ratio of ω-6 fatty acids to ω-3 fatty acids is about 0.1 to about 1.0.

16. The composition according to claim **15** in which said ω-3 fatty acids are present in a quantity of about 1.0 gm to about 100 grams per liter.

17. The composition according to claim **15** in which said ω-3 fatty acids are present in a quantity of about 5.0 grams to about 100 grams per liter.

18. The composition according to claim **15** in which said ω-3 fatty acids are present in a quantity of about 5.0 grams to about 10.0 grams per liter.

19. A liquid nutritional composition comprising:

a) at least 50 grams per liter of a source of amino-nitrogen, wherein 15 to 50% by weight of the amino-nitrogen is branched-chain amino acids, and wherein tryptophan is present in an amount less than about 5.0% by weight of the total amino-nitrogen;

b) an antioxidant component comprising about 2,500 to about 6,500 micrograms per liter of beta-carotene, about 250 to about 1,000 milligrams per liter of vitamin C, about 100 to about 500 I.U. per liter of vitamin E, and about 75 to about 125 microgram per liter of selenium, and;

c) at least 4.47 grams of eicosapentaenoic acid per liter.

20. A liquid nutritional composition comprising:

a) an antioxidant component comprising about 2,500 to about 6,500 micrograms per liter of beta-carotene, about 250 to about 1,000 milligrams per liter of vitamin C, about 100 to about 500 I.U. per liter of vitamin E, and about 75 to about 125 microgram per liter of selenium, and;

b) at least 4.47 grams of eicosapentaenoic acid per liter.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,077,828
DATED : June 20, 2000
INVENTOR(S) : Bonnie C. Abbruzzese, Mark A. McCamish, Frederick O. Cope, Stephen J. DeMichele

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

<u>Title page,</u>
Inventors, the name of the last inventor is spelled correctly, but the "m" in DeMichele should be capitalized.

<u>Claims,</u>
<u>Claim 6,</u>
Should read: The composition in claim 5 in which said tryptophan is present in an amount of less than 3.0% by weight amino-nitrogen.

Signed and Sealed this

Second Day of October, 2001

Attest:

Nicholas P. Godici

NICHOLAS P. GODICI

Attesting Officer *Acting Director of the United States Patent and Trademark Office*

EXHIBIT E

United States Patent [19]

Roberts

[11] **4,112,123**

[45] **Sep. 5, 1978**

[54] **NUTRITIONALLY BALANCED SINGLE FOOD COMPOSITION AND METHOD OF PRODUCTION**

[75] Inventor: **Willard Lewis Roberts,** Mequom, Wis.

[73] Assignee: **Beatrice Foods Co.,** Chicago, Ill.

[21] Appl. No.: **707,247**

[22] Filed: **Jul. 21, 1976**

[51] **Int. Cl.**^{2} **A23C 21/00**

[52] **U.S. Cl.** **426/72;** 426/74; 426/583; 426/801; 426/658

[58] **Field of Search** .... 426/810, 656, 658, 657, 426/72, 74, 532, 579, 590, 654, 613, 556, 583; 260/112 R; 424/177, 359

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Primary Examiner—Hiram H. Bernstein
Attorney, Agent, or Firm—Murray and Whisenhunt

[57] **ABSTRACT**

There is provided a single nutritionally balanced food composition for oral ingestion and producing low residues and diminished stoolings for use with patients having abnormal catabolic states. The composition comprises a water soluble or suspendible, essentially undenatured protein obtained from the ultra-filtration of whey and containing beta lactoglobulin, alphalactalbumin, immunoglobulins, and serum albumin; and medium-chain triglycerides of predominately 6 to 10 carbon atoms in the fatty acid chain. The composition will also include digestable carbohydrates, e.g. dextrose, sucrose, corn syrup solids, etc., and a food grade emulsifier. The composition can provide up to three calories per cubic centimeter of solution that can be drip fed and has a low osmolarity. The composition has a Protein Efficiency Ratio (PER) which is at least 3.1 and more usually 3.2. The protein is essentially bland to the taste and the composition therefore may be flavored as desired. The composition may be provided in a dried or reconstituted form of either low viscosity for tube-feeding and sipping or high viscosities for simulated foods, e.g. custards, puddings, candies, fillings for sandwich cookies, et cetera.

25 Claims, No Drawings

NUTRITIONALLY BALANCED SINGLE FOOD COMPOSITION AND METHOD OF PRODUCTION

The present invention relates to a nutritionally balanced food composition which may be used for oral ingestion by patients having an abnormal catabolic state. More particularly, it relates to such food composition, and methods of production thereof, which are quite palatable, easy to digest, can be variously flavored, and which provide high caloric value, with complete nutritional values, but additionally with low osmolarity.

BACKGROUND OF THE INVENTION

Certain patients develop abnormal catabolic states. This arises when the body metabolizes nutrients at a greater rate than the nutrients are supplied to the body, which results in a state of destructive metabolism, also referred to as abnormal metabolism. This state can be induced by illnesses, particularly those illnesses which interfere with normal digestion. Often, this state is caused by surgery which is disruptive of normal metabolism processes. Further, the state can be induced by traumas which induce a necessity for high caloric intake. For example, a burn patient may require as many as 7,000 calories per day due to the damage to the body and the results thereof occasioned by the burn.

With patients having an abnormal catabolic state, it is a constant problem to provide the required nutrients to that patient due to the difficulties encountered as a result of a specific malady or trauma. Very often this difficulty involves the inability of the patient to masticate natural foods or to digest natural foods. Further, in some patients, the use of natural foods produces too much residue for the patient to handle, due to disruptions of the digestive track by way of disease, trauma, or surgery. In the case of burn patients, attempting to simply digest 7,000 calories of natural foods it is an extremely difficult requirement for the patient.

As a result thereof, such patients often suffer severe body weight losses during these abnormal catabolic periods, resulting in severe complications to the primary malady and often resulting in severe body damage or even in death.

To regulate the problems associated with abnormal catabolic states, the art has proposed a variety of procedures. One procedure is that of intravenous feeding, since this route does not require digestion of the nutrients and thereby avoids problems with the digestive tract. While this approach is quite successful, it does have several limitations. Thus, the total volume of liquids which can be induced into the circulatory system is limited, and if attempts are made to over load the volumetric capacity, complications of the lungs and kidneys may well result. For this reason, it is often difficult to supply the required nutrients by venous routes simply by reason of volumetric limitations of the circulatory system. Additionally, it is most dangerous to introduce fats and oils into the blood stream, and intravenous procedures have, of necessity, eliminated fats including such essential fats as linoleic acid. Also, intravenous administered sugars, e.g. dextrose, contain only about half the calories on a weight basis as common fats, and it is difficult to provide sufficiently high caloric value by sustained intravenous feeding when an abnormal catabolic state exists.

To avoid the difficulties with intravenous feeding, a variety of orally ingestible compositions has been proposed. These compositions, generally, are based on the idea of providing a correct balance of protein, or amino acids, fat, carbohydrate, vitamins, and minerals. These systems do enjoy a reasonable success, but they also impose limitations on their use. The systems prepared from some proteins are of extremely high viscosities at high protein levels and, therefore, limit the applicability to those patients who can adequately swallow high viscosity compositions. Additionally, these compositions have utilized protein, protein hydrolysates, or amino acid sources in order to avoid the problem of the digestion of natural foods. The systems prepared from protein hydrolysates or 1-amino acids possess objectionable flavors that make it difficult, and in many cases impossible, for a patient to consume such products other than by tube feeding. Among the sources of nitrogen used are such sources as milk derived proteins, such as milks, casein, and the caseinates; vegetable derived proteins, such as soybean protein, protein hydrolysates, amino acid mixtures and the like. Milks as the source of protein contain high levels of lactose. Because some patients may be lactose intolerant it is desirable that foods of this type be relatively free of lactose. The proteins and protein hydrolysates used, unless supplemented with the proper essential 1-amino acids, may have relatively low Protein Efficiency Ratios. In the cases of some proteins it is difficult to formulate compositions with sufficient nitrogen to provide a high calorie, nutritionally balanced composition, while at the same time avoiding the difficulties of high viscosity. The systems prepared from protein hydrolysates or 1- amino acids possess objectionable flavors. Protein hydrolysates and mixtures of 1-amino acids contain the low molecular weight amino acids. These low molecular weight constituents, in combination with low molecular weight sugars and mineral compounds, used in the formulations, produce in the intestines a high concentration of low molecular weight compounds. As is known, a high concentration of such compounds induces a condition in the intestines where the osmotic pressure across the gut wall increases such that the body expells significant quantities of liquid into the intestines in an effort to wash out the high concentration of low molecular weight compounds. Thus, these compositions with high contents of low molecular weight amino acids, sugars, and salts so increases the osmotic pressure across the gut wall as to produce a condition called "dumping". The possibility of this condition is especially high with patients who require a low residue diet, because of complications in the digestive system. High osmolarity induces diarrhea in the patient as well as other related complications. The problem of high osmolarity is particularly accute in conventional compositions which rely upon protein hydrolysates and 1-amino acid mixtures as the primary nitrogen source, since the amino acids in the protein hydrolysates and 1-amino acid mixtures are of particularly low molecular weight and produce compositions which are particularly prone to induce "dumping".

Accordingly, it would be of distinct advantage in the art to provide compositions which avoid the problem of both intravenous feeding and feeding with conventional ingestible compositions as discussed above.

OBJECTS OF THE INVENTION

Accordingly, it is an object of the invention to provide ingestible nutritionally balanced food compositions which provide low residues and hence, diminished stooling, for use with patients having abnormal catabolic states. It is a further object of the invention to provide such compositions with high caloric content, while at the same time providing low osmolarity. It is a further object of the invention to provide such compositions with balanced amino acid source, and including fats, carbohydrates, vitamins, and minerals. It is yet a further object of the invention to provide such compositions with a protein having a high Protein Efficiency Ratio. Finally, it is an object of the invention to provide such compositions in a shelf-stable dried form, wich can be easily reconstituted to a relatively bland taste and hence, can be further formulated with flavors to provide dietary variety. Other objects will be apparent from the following disclosure and claims.

BRIEF DESCRIPTION OF THE INVENTION

The present invention is based upon the primary discovery that a particular protein presents a high nutritional profile but that profile does not include significant amounts of low molecular weight protein break down products, and thus formulations may be provided which avoid the problems of high osmolarity. Additionally, it has been discovered that the protein can be compounded with medium-chain triglycerides and carbohydrates into an emulsified product with a relatively low viscosity. This allows the composition to be used as a "tube-feeding" or drip composition, where the patient is not capable of significant mastication or has difficulty swallowing. That low viscosity also allows compounding with relatively high amounts of fats and carbohydrates to produce a composition with high caloric content.

The protein of the present invention may be described as a water soluble or suspendible, essentially undenatured protein obtained from the ultra-filtration of sweet or acid wheys and contains beta lactoglobulin, alpha lactalbumin, immunoglobulins and serum albumin.

Thus, the invention provides nutritionally balanced food composition for oral ingestion which produces low residues and diminished stooling for use with patients having abnormal catabolic states comprising from 3.5% to 27% of the above described protein, from 5% to 20% of medium-chain triglycerides of predominantly 6 to 10 carbon atoms in the fatty acid chain, from 50% to 75% carbohydrate and from 0.1% to 5% of a food grade emulsifier, all percentages being by weight on a dry basis. The resulting compositions can be used to provide solutions containing any concentration up to about three calories per cubic centimeter. Examples of useage are 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 calories per cubic centimeter. The rate of absorption and the osmolarity can be controlled by the carbohydrate used. For example low molecular weight carbohydrates, such as dextrose and sucrose, can be used when rapid absorption in the "brush border" is desired. Such solutions possess high osmolarity and should be "drip fed". High molecular weight carbohydrates, such as Low Dextrose Equivalent, high oligosaccharide content corn syrup solids can be used to produce solutions for tube or sip feeding. Such solutions possess osmolarities as low as 400 mOsm per liter of one calorie per cubic centimeter solution. Such solutions, fed at the proper rate, can be consumed without "dumping".

The Protein Efficiency Ratio of the protein is at least 3.1 and more often at least 3.2. The compositions can be compounded with the essential vitamins and minerals to supply the established Recommended Daily Allowance (RDA), or higher, based on a daily feeding of 2,000 calories.

The composition can be provided in a dried form which is easily reconstituted with water and may have such a low viscosity that drip feeding by a tube even as small as a standard size pediatric-naso-gastric tube is possible. Alternately, the dried form or reconstituted composition can be flavored to produce a sippy drink, or a more firm food such as a custard, a candy, and the like.

DETAILED DESCRIPTION OF THE INVENTION

The composition of the present invention is designed to be used as a total, nutritionally balanced single food composition, i.e. no other food for nutritional purposes is required. The total daily intake may be spread over 3, 4, 5 or 8 feedings or continuous drip, as desired, and as comfortable to the patient. The composition is based on a 2,000 calorie per day intake so that patients who do not require the higher caloric value will have a nutritionally balanced diet without undue body weight gain. However, at this level of feeding, the composition provides low residue and diminished stooling so that patients with digestive complications may easily maintain a sustaining diet with the present composition. The formulation also allows the composition to be produced in a viscosity which is sufficiently low that tube-feeding of the composition may be used. It can be drip fed at concentrations as high as 3 cal./c.c. On the other hand, if it is required to supply more than 2,000 calories per day, e.g. for burn patients, this extremely high caloric content can be achieved while yet providing a reasonably low residue. As can be appreciated, a diet consisting of 7,000 calories of natural foods of conventional compositions would produce extremely high residues and often result in complications of the digestive system.

Of critical importance to the present invention is the specific protein described above. This protein is obtained by the ultra-filtration of whey. This protein contains relatively low levels of lactose, lactic acid, and soluble salts present in whey. Other lower protein concentrate fractions derived by the ultra-filtration of whey, containing various levels of lactose, lactic acid, and soluble salts have been used as a skim milk replacer, an extender for frozen desserts, a bulking agent for dairy products, and in related applications.

The possibility of using whey protein as a substitute for natural protein has been recognized in the art. Thus, in the U.S. Pat. No. 3,935,323 to Feminella, the use of whey protein in place of egg whites in a whippable composition is acknowledged. The Feminella patent teaches heating the whey protein, in solution, to at least 90° C to produce a composition which can be whipped for up to about 8 hours after the heating.

The present protein fraction derived by ultrafiltration should be clearly distinguished from the heated protein of the Feminella patent. The present protein fraction is that which is retained by a semi-permeable membrane which allows passage of lactose, lactic acid, soluble salts, and low molecular weight compounds such as

amino acids and urea. Membranes of this nature may be either synthetic polymers or cellulosic membranes. Ultra-filtration membranes are made by the following companies: Abcor, DDS (labeled Hyper-filtration membrane), Union Carbide, Romicon, Dorr-Oliver and Universal Oil Products (Fluidcides Division). All of these membranes are acceptable for producing the present protein fraction.

The present whey protein fraction is characterized in that it is essentially undenatured, or at the most not denatured to the extent that insoluble protein will settle out from the prepared solution. It is also characterized in that the dried protein can be reconstituted in water to provide an opaque liquid, which is not a normal property of denatured whey protein. The protein fraction is also water soluble (or forms a stable suspension).

The protein concentration of the solids obtained by ultra-filtration can vary from as low as 12% to as high as 95%. However, where lactose and soluble salts contents, are of no concern, the protein content is of no concern. In order to maintain a significantly low lactose content the protein content should be at least 70%, and usually at least 80% of the solids content.

Thus, for purposes of the present specification, the term "ultra-filtration whey protein" is hereby defined to mean that water soluble or suspendible, essentially undenatured protein fraction derived from cheese whey which protein fraction is, essentially, retained by an ultra-filtration membrane that permits lactose, lactic acid, and soluble salts to pass through the membrane.

It should also be clearly appreciated, however, that the present protein is specific and identifiable in terms of its composition and is not, necessarily, dependent upon a process for production thereof. Thus, the protein with the present amino acid profile may be obtained by methods other than ultra-filtration, e.g. gel filtration, and the specification and claims should be so construed.

The amount of protein used in the present composition may vary widely, but for most applications from 8% to 20% on a dry weight basis is suitable, especially between about 10% and 16% and particularly about 14%.

It is emphasized that the protein must be essentially undenatured and water soluble or suspendible, since it must be capable of being compounded or formulated into a completely stable and pourable form in order to function in the manner required. Further, it must be the protein fraction containing beta lactoglobulin, alpha lactalbumin, immunoglobulins, and serum albumin normal to whey proteins separated by ultra-filtration. The percentages of these four proteins are not critical. The usual and preferred percentages are: beta lactoglobulin 55–67% (e.g., 62%); alpha lactalbumin 13–24% (e.g., 18%); immunoglobulins 12–14% (e.g., 13%); and serium albumin 6–7% (e.g., 6.5%).

While the amino acid profile of the protein fraction may vary, Table 1 below shows a typical amino acid profile for the ultra-filtration whey protein of the present invention. That table also compares the FAO/WHO suggested amino acid pattern with the present protein as well as the amino acid profile for whole egg protein. Further, to show the difference between the present ultra-filtration whey protein and the protein normally associated with milk, i.e. casein, the amino acid profile for casein is also shown in that table. Note that the present ultra-filtration whey protein amino acid profile follows very closely that of whole egg protein, while the amino acid profile of casein is substantially different from both.

Table 2 compares the present ultra-filtration whey protein with whole egg protein, casein, and soy protein isolate in terms of the grams of protein needed to meet standard requirements, and in terms of the Protein Efficiency Ratio (PER). Note specifically that the present ultra-filtration whey protein compares very favorably with whole egg protein in each category. Since the sulfur amino acids are the limiting amino acids in whey protein the quality of the protein can be improved by the addition of methionine, e.g. to raise the methionine from 2.5 to 3.7. This produces a protein superior to egg protein (See Table 3). Since whey protein contains such high levels of the essential amino acids it is an excellent base for the preparation of foods containing amino acid patterns that benefit patients with various diseases. For example, a whey protein-essential amino acid mixture possessing FAO/WHO essential amino acid pattern can be prepared by the addition of the proper levels of each essential amino acid. Such a food would be useful for kidney disease patients who must limit their nitrogen intake. Another example would be a food for liver disease patients requiring high levels of isoleucine, leucine, and valine and low levels of phenylalanine and methionine. Still another example would be a food meeting FAO/WHO essential amino acid pattern except for a low phenylalamine content. Such a food would be of value for feeding patients with phenylketonuria. Another example would be supplementation of the whey protein with a soluble or suspendible milk co-precipitate and the desired essential amino acids to produce a lower cost high quality protein for long term feeding.

It should also be appreciated that the ultrafiltration whey protein, being derived from cheese whey, may contain small amounts of cholesterol and fat. A typical analysis of the ultra-filtration whey protein is shown in Table 4.

The medium-chain triglycerides of the present invention form an important and critical feature of the present invention. These forms of fat produce compositions of low viscosity while at the same time provide high caloric content and easily digestible compositions. MCT passes directly to the liver via the portal vien and is metabolized somewhat like carbohydrates. Long chain fatty acids pass through the digestive tract and back to the liver via the lymphatics. Suitably, the fatty acid chain of the medium-chain triglycerides will be predominantly between about 6 and 10 carbon atoms.

The proportion of medium-chain triglycerides in the composition can vary widely, but between about 5% and 20% by weight may be used. More often, however, the proportion will be about 10% and 18%, with 16% being an optimal value.

Any food grade emulsifier may be used for present emulsification purposes and, indeed, combinations of emulsifiers may be used if desired. For example, any of the long fatty acid glycerol emulsifiers may be used, which normally have a C-12 to C-20 esterified chain. Typically among these are glycerol-lacto-palmitate or the stearate, etc. Alternatively, propylene derived emulsifiers may be used, such as propylene glycol-monostearate, or the oleate, palmitate, myristate, et cetera. Likewise, the "Span" series of emulsifiers may be used. These are well-known emulsifiers and are fatty acid partial esters of sorbitol anhydrides (or sorbitan). A preferred emulsifier is the "Tween" series, which is well known to the art, and are polyoxyethylene derivatives of fatty acid partial esters of sorbitol anhydride. Typi-

cally, Tween 80 and Atmos 300 are used, for their overall combination of properties, and this is a preferred embodiment. Also, other of the well known Atmos series of mono and diglycerides may be used. Also, lecithin may be used as an emulsifier. While the amount of emulsifier will be chosen to suit the particular composition, this will generally range from about 0.1% to 5% by weight, although usually this percentage will be between about 0.5% and 3%.

The composition will also contain from 50% to 75% by weight of carbohydrates, but more often between 55% and 70%. The carbohydrates may be any of the digestable carbohydrates such as dextrose, fructose, sucrose, maltose, oligosaccharides, higher saccharides, et cetera, or mixtures thereof, depending on usage.

With compositions as described above, the caloric content of solutions can be adjusted to any desired level up to about 3 calories per cubic centimeter.

Also, with this composition the osmolarity can be varied. If desired, this value can be decreased to as low as 450 or even 400 mOsm per liter of 1 calorie per cubic centimeter of food.

Vitamins, minerals, and other trace elements can be added to the composition as desired, but for purposes of total nutritional balance, these additions should be equal to the RDA or greater based on 2,000 calories. Table 5 shows a comparison of the nutrients in a 2,000 calorie feeding of the present composition and compares those nutrients with the standard of a 2,800 calorie feeding. Additionally, the percent of the RDA provided by the present composition is shown. Of course, it is not necessary that additional vitamins and minerals be provided in the present composition, and those small amounts can be given by way of a supplemental pill or injection, if desired. Nevertheless, the preferred embodiment of the invention includes those additional vitamins and minerals. The amount of vitamins in any unit of the present composition may be chosen so that the total number of units of the composition which will be taken by a patient in a single day will supply the total desired vitamins and minerals, e.g. the RDA or higher. However, the desired daily amount may be given in one unit.

The composition is advantageously provided in a powdered form of relatively low moisture content, e.g. at least below 4% by weight and more preferably below 3% by weight. This will provide an exceptionally long shelf-stable product, e.g. at least one year shelf-stability at ambient conditions if hermetically sealed.

Conventional coloring agents, such as the USDA colors, may be used, as well as conventional preservatives, such as BHT, BHA, citric acid, et cetera.

In addition to a powdered form, emulsified liquid forms may be prepared from the same formulations used for the preparation of the dried forms. The liquid form must be pasteurized or stored under refrigerated conditions.

The dried powder may be reconstituted with any desired edible liquid which will, essentially, solvate the powder. Thus, while it is possible to reconstitute the composition with liquid such as alcohol, absent some unusual circumstances, the reconstituting liquid will be principally water. The water may contain additional ingredients, for example, alcohol, glycerol, propylene glycol, sugars, flavors and the like for the functionality imparted by those additional compositions and compounds.

The composition may also include edible acids and bases, such as acetic acid, citric acid, lactic acid, potassium hydroxide, sodium hydroxide, calcium hydroxide, ammonium hydroxide, and the like, in order to adjust the pH within the range of 3.0 to 8.0and especially 6.5 to 7.0. Conventional buffers may also be used if desired, although the same is not necessary.

While the dried form of the invention is shelfstable, once the dried powder has been reconstituted, it must be stored under refrigeration and should be used within about 24 hours. By careful selection and processing, however, the dried composition will remain storage stable without significant degradation for one year or more. As can be appreciated, this is a very convenient form of the invention and allows hospitals and the like to conveniently store the composition and reconstitute the same as needed for preparing the foods.

In this latter regard, the dried powder is reconstituted simply by mixing with water or other liquid as described above. The ratio of water to the composition will, of course, vary with proportions of the ingredients of the composition, as discussed above, and with the desired consistency required.

Generally speaking, however, on a weight/weight basis of composition to water, the dilutions on a one liter basis will be:

To make 1000 ml (approx 34 oz)		
Calories/ml	Exact gms powd/ml water	Approx oz powd/fl oz water
½	113.5/940	4/32
1	227 /840	8/28
1 ½	340 /760	12/26
2	454 /640	16/22
2 ½	568 /520	20/18
3	682 /400	24/14

The preferred methods for drying ingredients or compositions containing whey protein are those using relatively low temperatures in order to avoid any substantial denaturization of the protein, e.g. spray drying or freeze drying in conventional manners. However, if spray drying, the air inlet temperature should be less than 350° F (e.g. less than 300° F) and the air outlet should be less than 180° F (e.g. less than 170° F) to insure quick but cool drying of the composition.

The invention will be illustrated by the following examples, but it is to be understood that the examples illustrate only the preferred embodiment of the invention and that the invention is not restricted to that preferred embodiment but extends to the scope of the foregoing disclosure. This preferred embodiment is in connection with proportions and ingredients, the protein portion of which closely approximates the nutritional values of natural egg. As noted above, however, it is not necessary that the composition be so formulated. In the examples, all proportions are by weight, unless otherwise specifically noted.

EXAMPLE 1

The single nutritionally balanced food composition, in powder form, is prepared by blending a number of ingredients. Ingredient (A), a spray dried powder contains the whey protein, fats, emulsifiers, and antioxidants. Ingredient (B), a spray dried powder consists of encapsulated and embedded minerals. Ingredient (C) is carbohydrate. Ingredient (D) consists of encapsulated vitamins.

Preparation of Ingredient (A)

3870 lbs of U.F. whey protein concentrate prepared from acid whey containing 449 lbs. protein (561 lbs solids) is batch pasteurized 30 minutes at 150° F. or HT-ST pasteurized 16 seconds at 165° F. and promptly cooled to 80° F. The pasteurized mixture is placed in a 600 gallon processing tank and neutralized to a pH of 6.7 with 21.5% KOH solution. An oil mixture of the following ingredients is prepared:

Medium-chain triglycerides. Predominantly C_6 and C_{10}: 534.9 lbs.
Safflower Oil (high linoleic acid type): 126.4 lbs.
Atmos 300 (an emulsifier): 28.4 lbs.
Tween 80 (an emulsifier): 7.1 lbs.
Tenox 7 (an antioxidant): 0.243 lbs.

This oil mixture is placed in the neutralized protein solution and mixed thoroughly. The mixture is then homogenized at about 1500 psi and spray dried through a 72-16 spray nozzle into a conventional box spray dryer. The temperature at any point ahead of the spray nozzle should not exceed about 100° F. The spray drier inlet air temperature is 300° F. The spray drier outlet air temperature is 170° F. The moisture content of the dry powder is approximately 3% by weight.

PREPARATION OF INGREDIENT (B)

The following ingredients are mixed with water, pulverized, and spray dried in a manner similar for (A):

Mineral Compounds: 7.90 lbs.
Maltrin-10 (hydrolyzed cereal solids): 3.11 lbs.
Gelatin (250 Bloom): 0.48 lbs.
Gum Arabic: 0.48 lbs.
Water: 16.0 lbs.

PREPARATION OF FINAL FOOD POWDER

The following ingredients are compounded by thorough blending:

Spray Dried Powder* (A)	260.8 g.
Clin Dri 1236*	329.9 g.
Sucrose*	57.7 g.
Spray Dried Powder (B)	29.5 g.
Vitamin Mixture	4.9 g.
	682.8 g.

*Carbohydrate Pattern of the three ingredients. 10.1% dextrose, 8.6% maltose, 7.2% trisaccharides, 6.5% tetrasaccharides, 5.8% pentasaccharides, 4.3% hexasaccharides, 32.1% higher saccharides, 23.7% sucrose and 1.7% lactose.

The 682.8 grams is divided and packaged into six 113.5 g, (4 oz.) packets. Each packet provides 500 calories. Thus 4 daily servings of the reconstituted 4 oz. packets provides the daily requirements of protein, carbohydrates, fat, vitamins and minerals and 2000 calories.

The percent calories from the various ingredients are: protein 12.5%, carbohydrates 49.2%, and fat 38.3%. Linoleic acid contributes 5.1% of the total calories. The osmolarity of the reconstituted composition on the basis of 1 calorie per cubic centimeter is 432 mOsm per liter.

EXAMPLE 2

Composition Example 1: 100 lbs.
Florasynth #74 Strawberry Flavor: 0.6 lbs.
Hercules Light Red Shade: 0.0075 lbs.

227 g. of the above flavored and colored powder is osterized with 320 c.c. water to produce 500 c.c. of 2 cal/c.c. drink.

* high-speed, high shear mixing

EXAMPLE 3

Baked Vanilla Custard (2 cal./c.c.)

Composition Example 1: 114 g
Water: 16 oz.
Vanilla: 1 tsp.
Nutmeg: dash
Mixed and baked at 325° F until firm

EXAMPLE 4

(B) Canned Puddings

Composition Example 1: 250 g.
Strawberry Flavor-Red Color: 0.75 g.
Red Color: 0.0315 g.
Water: 220 g.

The above is osterized and 230 g. per can is placed in 211 × 300 cans and heated to 160° F. The cans are seamed and cooked in boiling water for 20 minutes.

EXAMPLE 5

Quick Puddings

Composition Example 1: 80 g.
Gelatin (250 Bloom): 1.25 g.
Water: 69 g.

The gelatin is dissolved in 130° F. water and powder is added. Mixture is osterized and cooled in refrigerator about 2 hours.

EXAMPLE 6

Candy

Composition Example 1: 331 g.
Water: 35 g.
Lemon flavor-Yellow color to taste

The mixture is rolled in Amerfond (sucrose) powder and the pieces are coated with vanilla flavor bar chocolate.

EXAMPLE 7

Sandwich Cookies

Same as for candy except use 40 g. water
The mixture is spread between cookies.

EXAMPLE 8

Curdled Dessert

Composition Example 1: 212 g.
Water: 345 g.

The mixture is brought to a simmer in a pan with stirring to produce a fine cured and cooled in a refrigerator.

TABLE I

AMINO ACID CONTENTS OF U.F. WHEY PROTEIN, WHOLE EGG PROTEIN AND CASEIN				
AMINO ACID	FAO/WHO** SUGGESTED PATTERN g.	U.F. WHEY PROTEIN g. AMINO ACIDS g./100 PROTEIN	WHOLE EGG PROTEIN g. AMINO ACIDS g./100 PROTEIN	CASEIN g. AMINO ACIDS g./100g PROTEIN
Isoleucine	1.8	5.9	7.6	6.3
Leucine	2.5	13.0	9.7	9.6
Lysine	2.2	10.7	7.3	8.5
Methionine }		2.5	3.6	2.9
Cystine }	2.4	4.2	2.6	0.35
Phenylalanine }		4.3	6.3	5.2
Tyrosine }	2.5	4.1	4.9	6.5
Threonine	1.3	5.9	5.8	5.2
Tryptophan	0.65	2.5	1.9	1.8
Valine	1.8	6.2	8.6	7.5
Histidine		2.5	2.8	3.2
Arginine		3.4	7.4	4.3
Glycine		2.3	4.0	2.0
Aspartic Acid		12.0	7.4	7.4
Glutamic Acid		18.4	13.6	23.3
Proline		6.3	4.8	11.1
Serine		5.2	9.3	6.6
Alanine		5.6	7.4	3.3
	15.15	115.0*	115.0	115.05
PER		3.3	3.2	2.5

*The potential yield of amino acids from 100g. of each of the proteins is approximately 115g.

**World Health Organization Technical Report Series No. 522. Energy and Protein Requirements. Report of a Joint FAO/WHO Ad Hoc Expert Committee. - Table 17. Estimated Amino Acid Requirements of Adults. Page 55.

TABLE 2

U.F. WHEY PROTEIN, WHOLE EGG PROTEIN, AND SOY PROTEIN ISOLATE RATINGS				
	Grams Protein To Meet Requirements		ESSENTIAL AMINO ACIDS Per 100g. PROTEIN	
PROTEIN	Rose's-MDR(1)	FAO/WHO(2)	g.	PER
U.F. Whey Protein	16.7	35.8	59.2	3.3
Whole Egg Protein	17.7	38.7	58.3	3.2
Casein	34.0	73.8	53.85	2.5
Soy Protein Isolate	39.5	—	46.7	1.8

(1)Protein required to meet Rose's Minimum Daily Requirements.

(2)Protein to match 100 g Protein with FAO/WHO Suggested Amino Acid Pattern

TABLE 5

Nutrient	Unit	FDA RDA Per 2800 calories	Nutrients per 2000 Calories	RDA %
Ca	g	1.0	1.5	150
P	g	1.0	1.0	100
Na	g	*	0.6	*
K	g	*	2.5	*
Mg	mg	400	400	100
Fe	mg	18	18	100
Zn	mg	15	15	100
Cu	mg	2	4	200
Mn	mg	*	2	*
I	mcg	150	150	100
Cl	g	*	3.55	*
Vitamin A	IU	5000	5000	100

TABLE 5-continued

Nutrient	Unit	FDA RDA Per 2800 calories	Nutrients per 2000 Calories	RDA %
Vitamin D	IU	400	400	100
Vitamin E	IU	30	40	133
Vitamin K	mg	*	1.0	*
Vitamin C	mg	60	120	200
Thiamin	mg	1.5	1.8	120
Riboflavin	mg	1.7	2.0	117
Niacin	mg	20	20	100
d-pantothenic Acid	mg	10	10	100
Pyridoxin	mg	2.0	2.0	100
Folic Acid	mg	0.4	0.4	100
Vitamin B_{12}	mcg	6	6	100
d-biotin	mg	0.3	0.4	133
Choline	mg	*	80	*
Inositol	mg	*	16	*
Linoleic Acid	g	*	12	*

No RDA established.

TABLE 4

ANALYSIS OF WHEY PROTEIN SOLIDS	
Protein	80.0%
Fat	4.6
Lactose	5.8
Lactic Acid	4.6
Ash	4.4
Undetermined	0.6
	100.0%

TABLE 3

Amino Acid Content of U.F. Whey Protein (M). Whole Egg Protein. And Casein Estimated Relative Quality For Each Protein.									
	Fao/Who (18) Suggested Pattern	Fao/Who (18) Theoretical Protein Yields of Amino Acids		Whole Egg Protein (19-21) Yields of Amino Acids		U.F. Whey Protein[a] Plus Added l-Methionine Yields of Amino Acids		Casein (22) Yield of Amino Acids	
Amino Acid	& SD1 g	g/100 g Protein	g/28.7 g Protein	g/100 g Protein	g/38.7 g[b] Protein	g/100 g Protein	g/31.0g[b] Protein	g/100 g Protein	g/73.8g[b] Protein
Isoleucine	1.8	6.3	1.8	7.6	2.9	5.8	1.8	6.3	4.6
Leucine	2.5	8.7	2.5	9.7	3.8	12.8	4.0	9.6	7.1
Lysine	2.2	7.7	2.2	7.3	2.8	10.6	3.3	8.5	6.3
Methionine				3.6		3.7		2.9	
Cystine				2.6		4.1		0.35	
Meth. & Cyst.	2.4	8.4	2.4		2.4		2.4		2.4
Phenylalanine				6.3		4.3		5.2	
Tyrosine				4.9		4.1		6.5	
Phenyl. & Tyro.	2.5	8.7	2.5		4.3		2.6		8.6
Threonine	1.3	4.5	1.3	5.8	2.2	5.8	1.8	5.2	3.8
Tryptophan	0.65	2.3	0.66	1.9	0.74	2.5	0.78	1.8	1.3
Valine	1.8	6.3	1.8	8.6	3.3	6.1	0.9	7.5	5.5

TABLE 3-continued

Amino Acid Content of U.F. Whey Protein (M). Whole Egg Protein. And Casein Estimated Relative Quality For Each Protein.

Amino Acid	Fao/Who (18) Suggested Pattern & SD1 g	Fao/Who (18) Theoretical Protein Yields of Amino Acids g/100 g Protein	Fao/Who (18) Theoretical Protein Yields of Amino Acids g/28.7 g Protein	Whole Egg Protein (19-21) Yields of Amino Acids g/100 g Protein	Whole Egg Protein (19-21) Yields of Amino Acids g/38.7 g[b] Protein	U.F. Whey Protein[a] Plus Added 1-Methionine Yields of Amino Acids g/100 g Protein	U.F. Whey Protein[a] Plus Added 1-Methionine Yields of Amino Acids g/31.0g[b] Protein	Casein (22) Yield of Amino Acids g/100 g Protein	Casein (22) Yield of Amino Acids g/73.8g[b] Protein
Histidine		↑	↑	2.8	1.1	2.5	0.78	3.2	2.4
Arginine				7.4	2.9	3.5	1.1	4.3	3.2
Glycine		↑	↑	4.0	1.5	2.3	0.71	2.0	1.5
Aspartic Acid		62.1	17.8	7.4	2.9	11.9	3.7	7.4	5.5
Glutamic Acid		↓	↓	13.6	5.3	18.2	5.6	23.3	17.2
Proline		↓	↓	4.8	1.9	6.2	1.9	11.1	8.2
Serine				9.3	3.6	5.1	1.6	6.6	4.9
Alanine		↓	↓	7.4	2.9	5.5	1.7	3.3	2.4
		115.0[c]	32.96	115.0[c]	44.54	115.0[c]	35.67	115.05[c]	84.9

[a]1.1% of the total amino acids consists of added 1-methionine.
[b]g. Protein required to supply level of limiting essential amino acids in 28.7 g. Fao/Who theoretical protein.
[c]Analytical information calculated to a common basis for more accurate comparisons. Assumed 16g N/100 g protein in all cases. The potential yield of amino acids from 100 g protein is approximately 115 g.

What is claimed is:

1. A single food nutritionally balanced composition for oral ingestion and producing low residues and diminished stooling for use with patients having abnormal catabolis states consisting essentially of:

(a) from 8% to 27% by weight of water soluble, undenatured ultra-filtration whey protein;

(b) from 5% to 20% by weight of medium chain triglycerides of predominantly 6 to 10 carbon atoms in the fatty acid chain;

(c) from 50% to 70% by weight of carbohydrates selected from the group consisting of corn syrup solids, trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, dextrose, fructose, sucrose, maltose, oligosaccharides and higher saccharides;

(d) from 0.1% to 5% by weight of a food grade emulsifier;

and wherein the composition provides from 1 up to about 3 calories per cubic centimeter of composition.

2. The composition of claim 1 where the Protein Efficiency Ratio of the protein is at least 3.1.

3. The composition of claim 1 where the Protein Efficiency Ratio of the protein is at least 3.2.

4. The composition of claim 1 wherein the protein is bland to the taste.

5. The composition of claim 1 wherein the amino acid profile of the protein at least equals that of eggs.

6. The composition of claim 1 where the composition contains vitamins and minerals which supply at least the established RDA when the composition is consumed in amounts to provide 2000 calories per day.

7. The composition of claim 1 which also includes at least one member selected from the group consisting of, flow control agents and flavoring agents.

8. The composition of claim 1 in a liquid form.

9. The composition of claim 8 wherein the liquid form contains water.

10. The composition of claim 1 wherein a preservative is added to the composition.

11. The composition of claim 10 wherein the preservative is citric acid.

12. The composition of claim 1 wherein the cholesterol content of the composition is less than 0.1 mg. per gram of composition.

13. The composition of claim 1 wherein the pH of the composition is between 3.0 and 8.0.

14. The composition of claim 13 wherein the pH of the composition is between 6.5 and 7.0.

15. The composition of claim 1 in flavored and colored drink form.

16. The composition of claim 1 in baked custard form.

17. The composition of claim 1 in canned pudding form.

18. The composition of claim 1 in quick pudding form.

19. The composition of claim 1 in candy form.

20. The composition of claim 1 in sandwich cookie form.

21. The composition of claim 1 in curdled dessert form.

22. The composition of claim 1 in a dried form and having a shelf life of at least one year.

23. The product of claim 1 in a frozen liquid form.

24. The composition of claim 1 in the form of a low viscosity liquid.

25. The composition of claim 1 in the form of a high viscosity liquid.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,112,123

DATED : September 5, 1978

INVENTOR(S) : Willard Lewis Roberts

It is certified that error appears in the above–identified patent and that said Letters Patent are hereby corrected as shown below:

Column 10, line 15 of Example 3, change "Water: 16 oz." to -- Water: 160 g. --

Column 10, line 67, Example 8, change "cured" to -- curd --

On the title page in connection with Item No. [75] the Inventor, change the city of the residence of the inventor from "Mequom" to -- Mequon --

Signed and Sealed this

Twentieth **Day of** *May 1980*

[SEAL]

Attest:

SIDNEY A. DIAMOND

Attesting Officer

Commissioner of Patents and Trademarks

EXHIBIT F

US006420342B1

(12) **United States Patent**
Hageman et al.

(10) **Patent No.: US 6,420,342 B1**
(45) **Date of Patent: Jul. 16, 2002**

(54) **NUTRITIONAL PREPARATION COMPRISING RIBOSE AND MEDICAL USE THEREOF**

(75) Inventors: **Robert Johan Joseph Hageman**, Waddinxveen; **Rudolf Leonardus Lodewijk Smeets**, Venlo; **George Verlaan**, Wageningen, all of (NL)

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/566,381**

(22) Filed: **May 8, 2000**

(51) **Int. Cl.**[7] **A61K 31/70**; A61K 31/495; A61K 31/195

(52) **U.S. Cl.** **514/23**; 514/52; 514/249; 514/561; 514/565

(58) **Field of Search** 514/23, 52, 249, 514/561, 565

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(74) *Attorney, Agent, or Firm*—Young & Thompson

(57) **ABSTRACT**

Trauma, surgery, inflammation, subfertility, lactation problems, gut disorders, infant nutrition, cancer, arthritis and other joints problems, vascular problems and cardio- or cerebro vascular problems, ischaemia, aging, impaired immune function, burns, sepsis, malnutrition, problems with liver or kidneys, malaria, cystic fibrosis, migraine, neurological problems, respiratory infections, improvement of sports results, muscle soreness, drug intoxication and pain can be treated with a nutritional composition containing effective amounts of ribose and folic acid, optionally combined with other components such as niacin, histidine, glutamine, orotate, vitamin B6 and other components.

22 Claims, No Drawings

NUTRITIONAL PREPARATION COMPRISING RIBOSE AND MEDICAL USE THEREOF

The invention is related to nutritional, pharmaceutical or dietetic preparations that comprise ribose or folic acid or functional analogs thereof and the use of these compositions in the prevention or treatment of specific diseases that are related to disorders or insufficiencies of total nucleotide metabolism.

BACKGROUND OF THE INVENTION

Nucleotides are heterocyclic compounds that occur in all mammals. Nucleotides consist of a purine or pyrimidine base, a sugar unit and one to three phosphate groups. The major purine bases that occur in the human body are adenine (6-aminopurine), guanine (2-amino-6-hydroxypurine), hypoxanthine (6-hydroxypurine) and xanthine (2,6-dihydroxypurine); the major pyrimidines are uracil (2,4-dihydroxypyrimidine), cytosine (2,4-dihydroxy-5-methylpyrimidine) and thymine (4-amino-2-hydroxypyrimidine). The sugar moiety can be ribose (in ribonucleosides) or 2-deoxyribose. The sugar moiety is connected to the base through a β-N-glycosidic bond at N9 of the base; the phosphate groups are connected to the sugar moiety through the 3' or 5' position. When the phosphate groups are split from nucleotides compounds called nucleosides are formed.

For the purpose of this document, total nucleotide netabolism (TNM), is defined as the combination of all biochemical pathways in which nucleotides, their precursors and metabolites are directly involved as main ingredients and that occur in the body of mammals. The pathways include the synthetic routes for purines and pyrimidines, both de novo and salvage pathways, starting from carbamoyl phosphate and 5-phosphoribosyl-1-pyrophosphate (PRPP), respectively. They also include the interconversions of the various nucleotides into each other, the phosphorylation and dephosphorylation reactions of respectively nucleosides and nucleotides and the catabolic pathways of nucleotides to the compounds that are cleared from the body. They do not include the further reactions of phosphoric groups thus split off from the phosphorylated nucleotides.

Nucleotides and their related metabolites play a key role in life, as has been described in the biochemical literature. Triphosphorylated forms, and especially adenosine triphosphate (ATP), are the main forms of chemical energy in mammal's body. This type of energy is for example required to allow desirable biochemical reactions to occur at a substantial rate, to maintain ionic gradients over membranes and to allow transport of some important, components over membranes. Nucleotides also can provide phosphate in a lot of biochemical reactions Nucleotides (bases) can form building blocks for DNA and RNA. Nucleotides and their derivatives can serve as mediators or regulators of many metabolic processes; for example the cyclic form of the monophosphates of adenosine and guanosine function as a second messenger after activation of receptors in the membrane. Due to allosteric effects they regulate many pathways. ADP is involved in platelet aggregation. Adenosine is a potent vasodilator and receptors have been described for other nucleic acid bases as well. Nucleotides are also part of many key cofactors such as NAD, FAD and CoA.

Nucleotides also activate intermediates in many reactions. Interconversion reactions of monosaccharides require activation by means of various nucleotides; there monosaccharides form important constituents of glycoproteins. Ethanolamine also requires activation before it can be modified intocholine, and ATP is needed to activate methionine in order to have if function as a methyl donor.

Nucleotides or precursors thereof can be formed in the body or be consumed from the diet. Nucleotides from diet can be broken drown in the digestive tract to nucleotides and nucleic acid bases, which can be rapidly absorbed by the gut and can be reassembled to nucleotides and related metabolites. Xanthines occur widely in drinks and chocolate.

Nucleotides can be synthesized de novo in several tissues using a pathway that requires the presence of much energy (ATP) and many reactants. That is way the human body is equipped with salvage systems that allow effective reuse of catabolic products of nucleotides.

Under certain conditions, e.g. when an unbalanced diet was consumed or when (severe) tissue damage has occurred in a short period of time, the body is temporarily exposed to large amounts of nucleic acids. Pyrimidines are in this case catabolized to beta-aminoisobutyric acid (thymine) or beta-alanine (e.g. uracil) that can be cleared through the urine. Beta-alamine can also be used for biosynthesis of carnosine and anserine by reaction with histidine or 1-methyl histidine. Excess purines are metabolized to xanthine (2,6-dihydroxypurine) and finally uric acid (2,6,8-trihydroxypurine).

Uric acid is mainly synthesized in the liver and thereafter released in the circulation. In extra-cellular fluids (e.g., synovial fluid or blood plasma) it occurs in the ionized form. Normal levels in blood serum are three to 6–7 mg/100 ml. The latter concentration is similar to or above the solubility product of monosodium urate at 37° C., which indicates the risk for (local) precipitation of urate crystals. Urate is normally predominantly (>⅔) cleared via the urine.

Hyperuricemia is defined as that situation when serum urate is bove 7.0 mg/100 ml in men and above 6.0 mg/100 ml in women. The occurrence of hyperuricemia is associated with disorders like obesity, hypertension, alcohol abuse, and congestive heart failure, though it is not considered to be a cause of these disorders. Nevertheless hyperuricemia may lead to diseases like gouty arthritis, tric acid urolithiasis and even nephropathies and also occurs in the syndrome of Lesch-Nyhan. Therefore it is important to ensure that at all times urate levels in serum and urine remain at normal magnitudes.

Uric acid contributes under normal conditions significantly to the total antioxidant (radical scavenging) capacity of blood plasma. It has been reported that total antioxidant capacity can be important for detoxifying reactive species, such as free radicals, e.g. these that are released during uncontrolled inflammatory conditions, and toxic (exogenous) compounds. It is also reported that scavenging of free radicals is important to prevent damage to membranes of cells.

Compounds that are normally used as antioxidants such as ascorbic acid and tocopherols have to be administered in huge amounts in order to have them contribute to the same extent to the total antioxidant capacity of blood plasma. This would lead to undesirable side effects in the product, and, on the longer term, also in persons who consume such a product, due to the prooxidaant effect of these components. Administration of other redox-active compounds to meet the same antioxidant capacity at urate may lead to undesirable side-effects such as interaction with other circulating antioxidants/radical scavengers such as serum albumin.

Urate is normally produced from xanthine by the enzyme xanthine dehydrogenase. Under certain conditions these

enzyme is converted to xanthine oxidase. In this form the enzym uses oxygen as oxydant and hydrogen peroxyde is formed. It is important that the latter compound is neutralized before it can cause harm.

Therefore a need exists to develop a preparation that ensures a constant and sufficiently high antioxidant capacity of extracellular fluids such as blood plasma without undesirable side effects in both product and patients.

Due to their importance for life, nucleotides are rapidly metabolized and a high turnover rate exists. Some metabolites can interconvert using well-described pathways. There pathways are highly regulated and interdependent. Under normal conditions these pathways occur rapidly. This ensures a rather constant concentration of all nucleotides and/or related metabolites, whose magnitude depends on the requirements that are set by the condition of the various tissues and cells and on local concentrations of many components that are involved in TNM.

Under several conditions the human body is not able to maintain homeostasis of all nucleotides and related metabolites in all tissues or cells.

Several diseases have been associated with problems with nucleotide metabolism and in particular ATP levels of specific tissues. In ischaemic situations, tissue concentrations of ATP rapidly decrease and the same occurs in protein-energy malnutrition.

Therefore a need exists for nutritional preparation that ensures the presence of adequate amounts of nucleotides in the different tissues and in such a way that to imbalances occur between the amounts of the various metabolites.

The relevance of the presence of adequate concentrations of various nucleotides has been recognized in the field of infant formula, in particular to ensure development or maintenance of a proper gut function in the young infant. Specific amounts of various nucleotides or their metabolic equivalents are added to obtain a result. See for example WO 95/18618 and WO 95/18547. The composition of human milk is used as reference.

However, the amounts of nucleotides required by the various tissues under varying conditions have not been described in the art. This applies to the developing gut in the premature child, but also to tissue that has been damaged due to trauma, radiotherapy or surgery or for temporary local requirements of tissue that comprises rapidly dividing cells. Also it has not been known up to now how much of a daily oral dose of some milligrams per day is really available to individual patients. Administration of nucleotide mixtures may thus easily result in either ineffective dosages or overdosages of specific nucleotides or metabolites thereof. Imbalances with regard to the amount or type of nucleotides or related metabolites may also easily disturb normal metabolic processes in cells, especially in sensitive cells like erythrocytes.

In manufacturing practice, it appears that nucleotides are expensive ingredients, which make it difficult to manufacture effective preparations at a reasonable price for the consumer.

For these reasons other components were sought that could be used in nutritional preparations and could influence nucleotide levels.

Glutamine, as important donor of amine groups, plays a role in nucleotides biosynthesis. Kovacevic demonstrated in 1987 that administration of glutamine together with inosine increased cellular ATP content. Administration of glutamine alone was not effective.

EP-A 0 540 462 discloses the use of L-glutamine or its functional equivalents for the treatment of a fall in blood L-glutamine level in people involved in endurance exercise, physical activity or suffering from overtraining.

D-Ribose is a pentose that occurs in many organisms. It is one of the metabolites of glucose when the latter is metabolized in the human body via the oxidative pentose pathway. This pathway yields pentose phosphates that can interconvert and yields glyceraldehyde 3-phosphate, which can be used as energy source.

Ribose can also be activated by ATP to 5-phosphoribosyl-1-pyrophosphate (PRPP), which is involved in the de novo biosynthesis, of purines and pyrimidines in a very energy (ATP)-demanding synthetic route and in salvage pathways of these bases.

Nutritional preparations which comprise ribose as single active component are available commercially and are used to increase body performance of sportsman.

WO 99/65476 describes the use per daily dose of 0.1–100 g of a pentose, preferably ribose, and preferably 1–20 g, optionally together with creatine, magnesium, carnitine and arginine to increase energy levels of an organism in vivo. In DE 19659755 Pliml discloses that 1–50 g/day ribose can be used to increase performance for sportsman. The combination of 0.2–2 g/day ribose and 0.1–1 g/day magnesium aspartate was disclosed by Palazzi in WO 92/15311.

Carniglia disclosed in U.S. Pat. No. 5,391,550 that oral administration of a product that comprises ribose, amino acids and a compound selected from the group choline, inositol and carnitine was effective in increasing intracellular ATP levels and for improving wound repair. Preferably the composition comprised 10–40 parts ribose, 30–60 g amino acids and 10–30 parts of either choline, carnitine or inositol or combinations thereof. The latter components are in our opinion not required to support TNM and are therefore not included in the products according to the invention.

U.S. Pat. No. 4,719,201 and U.S. Pat. No. 4,605,644 disclose the effect of ribose, optionally with adenine for improving recovery after ischaemia, when the compound is present in a solution for perfusing a tissue e.g. during surgery. In U.S. Pat. No. 4,880,783 a heart perfusate is disclosed which comprises adenosine, ribose and hyproxanthine and increases the period of time in which the heart is deprived from a normal oxygen supply during surgery.

None of these papers reveal beneficial effects from ribose other than the increase in intracellular ATP and improvement of wound healing. Also no measures have been disclosed to avoid too large amounts of ribose to be converted to nucleotides and uric acid. Neither a synergy in effect has been reported when folic acid or possibly other active components, is administered together with ribose, especially in those persons that the have consumed diet that is improper for their condition.

The prior art discloses that in some cases large amounts of ribose (up to 100 g) must be consumed to observe a beneficial effect. This poses significant problems to the person who suffers from a bad appetite or restrictions in the volume of diet that he/she can consume per day, such as the elderly.

Therefore a need exist for a nutritional preparation that is more effective in supporting total nucleotide metabolism in the body without causing the disadvantages of the prior art. Support of the total nucleotide metabolism should lead to an increase of the levels of all nucleotides and related metabolites in all tissues, especially those in need thereof, but only to optimal values. This should lead to the use as defined in

the next clause. Disadvantages associated with the use of prior art products are a high price and a significant risk on metabolic disturbances especially in sensitive cells by administration of higher amounts of specific nucleotides and the risk of hyperuricemia and associated diseases.

SUMMARY OF THE INVENTION

It has been found that a nutritional preparation that comprise ribose, or its functional equivalents, and folic acid, or its functional equivalents, supports total nucleotide metabolism in persons in need thereof. This support is achieved by optimally using the metabolic and regulating systems as present in the body of the mammal. This results in a situation that local shortages in supply of nucleotides or related metabolites are earlier restored and the risk on hyperuricemia is lower than when methods are used as described in the prior art. Ribose should be used in an amount o at least 0.5 g per daily dosage, up to about 40 g per daily dosage. Folic acid (folate) should be used in an amount of at least 100 μg/day, up to about 20 mg/day.

The components are food grade, non-toxic, have a natural origin, are readily available at a specified quality and a reasonable price and are not complicated to include in a wide range of dietetic or nutritional preparations. In addition they have a good taste.

In order to increase the effect of the preparations or to decrease the risk of imbalances in TNM, it is preferred, according to the invention, to include other components as well. The components can be magnesium, orotate, niacin, selenium, thiamin, vitamin B6, glucose, citrate, specific amine acids such as histidine or glutamine, phosphate, sulfate or vitamin B12, their equivalents and/or combinations thereof.

The compositions according to the invention can be used to regulate and support TNM total nucleotide metabolism. It has been found that this effect is advantageous in the prevention and treatment of a wide range of diseases, disorders and health problems. These include trauma, surgery, inflammation (either acute and chronic), subfertility, lactation problems, gut disorders, infant nutrition (jaundice, gut maturation), cancer, arthritis (osteo-, rheumatoid) and other joint problems, vascular problems and cardio-or cerebro vascular problems, ischaemia (imparted peripheral blood supply, infarcts), aging, respiratory infections, impaired immune function (such as after chemotherapy and during AIDS), burns, sepsis, malnutrition, problems with liver or kidneys, malaria, cyclic fibrosis, migraine, neurological problems such as Huntington, Parkinson, Alzheimer, schizophrenia and depression, improvement of sports results, music soreness, drug intoxication and pain.

The products are preferably fortified with components that are specific for the groups of patients for which the products will be used, as described in detail below.

DETAILED DESCRIPTION OF THE INVENTION

The nutritional, pharmaceutical or dietetic preparation according to the invention can be manufactured in dry form, for example as bar, as powder, as tablet, but cookie or as cereal. The preparations can also have a liquid form, e.g. as drink, served as ready to drink, as pudding, as sauce as capable or as (soup) concentrate.

The preparations are manufactured using methods that are known in the art. For example powders can be manufactured by spray-drying or drying on drums. Spray-dried powders can be agglomerated in order to modify bulk density or solubility performance (e.g., wettability). When powders are manufactured, methods for preventing sticking of the powder can be taken that are known in the art, e.g. the addition of specific agents such as tricalcium phosphate or silicon dioxide. When tablets are manufactured the usual tabletting agents (such as magnesium stearate) are included. In order to give the nutritional preparations optimal organoleptic properties, methods can be used that are known in the art, such as setting of pH and the addition of flavoring agents and colorants. Also preservatives can be added to increase shelf life, and whose use in known in the art.

Appropriate amounts of the ingredients are blended in order to manufacture the final product. The final product can also comprise separate compartments that each may comprise part of total number or amount of the ingredients that are required.

The final product can be packaged in a way that is suitable for the type of product that is selected. These ways are known in the art. For example liquids can be packaged in bottles or cartons, that have a volume that is appropriate for containing the volume that is required for 0.5–10 daily doses. Powders can for example be packed in cans, bags or sachets. These packages can have a volume of typically 0.5–50 daily doses.

The active components can be mixed with other ingredients that are suitable. For example powdered forms of the preparations according to the invention can use spray-dried proteins of dairy, vegetable or animal origin, such as skimmed milk powder, whey powder, egg white powder, potato protein, soy protein, etc. or hydrolysates, or mixtures thereof. It is preferred to use proteins that are relatively rich in histidine and glutamine and poor in tryptophan, such as caseins. Specific synthetic amino acids, such as L-histidine, or peptides, such as alanyl-gluamine or glutamyl-glutamine, may be added to achieve this goal.

When proteins are included in the nutritional preparations, the amount that is included depends on the application of the product. In complete formulae typically an amount of 5–120 g per daily dose is included. In complete formulae for young infants the amount will be in the range 5–15 g per daily dose and preferably 6–10 g per daily dose. In complete enteral nutrition for feeding surgery patients typically 50–120 g and preferably 60–90 g per daily dose are included.

In supplements typically 0–60 g protein per daily dose will be included. In supplements for sportsmen and persons that temporarily have high protein requirements (such as burn patients) or deficiencies in amino acid status (e.g. due to malnutrition) up to 60 g protein per daily dose can be included. In other situations protein is not included in the product or present in lower amounts, typically 0–20 g per daily dose. In supplements it is advantageous to include free amino acids, especially histidine, in particular the L-isomer. In some cases the support of TNM should be combined with support of growth and anabolism.

It appears that especially a mixture of histidine, the branched chain amino acids leucine and isoleucine, lysine, methionine and phenylalamine has anabolic properties. For example in products for sportsmen the following mixture of amino acids appeared to be especially beneficial for muscle growth, when consumed in an amount of more than 2 and preferably more than 4 g per daily dose: 3–10 wt % histidine, 5–15 wt % isoleucine, 10–23 wt % leucine, 10–23 wt % lysine, 5–15 wt % methionine, 5–15 wt %, phenylalamine

and 5–15 wt % threonine. The product should in such a case contain no or little protein.

When relative large amounts of proteins or amino acids are included in the product it is preferred to increase the amount of vitamin B6 in the product. The extra amount that should be included in the product can be determined by using the criterium: 2 mg extra vitamin B6 per 100 g protein equivalent. As vitamin B6 source, pyridoxine, pyridoxamine or pyridoxal or functional equivalents thereof can be used.

Also powdered carbohydrates can be used that should be able to serve as glucase provider. Glucose syrup (dried) or starches and especially their hydrolysates are useful. In sweet products it is preferred to use malto-dextrins that are heavily hydrolyzed. In more salty products lightly hydrolyzed maltodextrins should be used, preferably those having a degree of hydrolyses below twelve.

It is important to include glucose into the product, because it allows the biosynthesis of sufficient amounts of reducing equivalents and of chemical energy. Both are essential for total nucleotide metabolism. In addition glucose can neutralize excess ribose, which reaction is supported by thiamine phosphate and magnesium. From glucose also extra ribose can be formed in those situation that: (1) too little ribose is administered via the claimed products or (2) too little ribose is present in tissue, relative to the requirements that are put by the specific conditions of the tissue. It is therefore preferred to include in the product 1–50 g glucose or its functional equivalent and more preferably 2–20 g is included.

Also 0.4–10 mg, and preferably 1.0–8 mg thiamin per daily doses, or its functional equivalents should be included. Preferably the hydrochloric acid salt is used.

As source of the active components either pure chemical substances or their functional equivalents such as racemic mixtures or food grade extracts of raw materials or mixtures thereof can be used as ingredients.

D(−)Ribose is a pentose sugar that can be purchased as crystalline product. Ribose is also a main constituent of nucleic acids. These acids can be isolated from yeast or lives. Either the nucleic acid fraction or extracts of hydrolysates thereof can be used as ribose source. It is preferred to use a preparation that comprises a higher amount of ribose than of the sum of nucleotides, nucleosides and nucleic acid bases, due to the imbalances in endogenous nucleotide concentrations that could occur. It is also possible to use rough extracts from carbohydrate hydrolysates. For example potato starch can be converted by an enzymatic process into a ribose-rich ingredient. Most preferable ribose, when referred to in the specification, does not include nucleic acid bound ribose.

It is preferred to use synthetic ribose, either as racemate, but most preferably as D(−)ribose. When D(−)ribose is used as ribose source per daily about 0.5–40 g should be included in particular 1–15 g and most preferably 2–10 g. When other materials are used as ribose source it depends on the ribose content of the ingredient how much of that raw ingredient should be included in the composition.

Where reference is made in this specification to folic acid or folate, all functional equivalents of folic acid are included. Folate is a vitamin that can be purchased as free folic acid (pteroyl-monoglutamic acid) or folinic acid (formyl-tetrahydrofolic acid). Also polyglutamate forms can be used as functional ingredient, especially when also zinc is present in the formulation. Rich sources of folate are yeast and liver and extracts of some green vegetables (broccoli, brussels sprouts, spinach), or fruits (citrus fruit) on the condition they are standardized on folate content. It is preferred to use synthetic folic acid. In order to be effective the compositions should comprise at least 100 μg folate per daily dose. This limit is selected to support adequately total nucleotide metabolism in young infants. In adults the amounts that are required have to be higher; preferably more than 250 μg have to be supplied per daily dose and most preferably more than 400 μg. In those adults that suffer from a genetically a compromised enzyme-system to metabolise folate even higher amounts are required, e.g. more than 1 mg/day. For normal adults it is most preferred to include maximally 5 mg folate in the product. When amounts above 4 μg folic/folate per kg body weight are supplied per day, it is preferred that at least 1 μg vitamin B12 per 100 mg folate is included.

To observe an optimal effect it is required to include niacin into the product. Niacin appears to have a synergistic effect when supplied together with ribose and folate, with regard to support of TNM and the formation of DNA and RNA. At least 4 mg niacin equivalents (=NE) should be included, in products for adults in particular 30–200 mg NE per daily dose. It is thought that in this dose niacin provides in many persons extra reducing equivalents to diseased tissue to allow increased production of chemical energy and conversion of different forms of nucleotides and of folates and increase formation of DNA and increase tissue levels of triphosphorylated forms of all nucleotides.

It is also preferred to include magnesium in the formulae. It appears that magnesium deficiencies occur rather frequently. Deficiencies will cause a decreased capacity to transfer activated phosphates to other molecules. Magnesium should be present in an amount of at least 20 mg per daily dose. Products for adults comprise preferably per daily dose (dependant on the application of the product) 100–500 mg.

It is important to include several compounds that are able to influence rates of desirable metabolic patterns. For this reason the product should preferably include histidine. Histidine is also added to neutralize excess beta-alanine and permit biosynthesis of sufficient carnoserine and anserine. Typically the amount of histidine will be about 0.2–5.0 g per daily dose and preferably 0.3–3 g per daily dose. Preferably glucose is also present when histidine is present, with a histidine to glucose weight ratio of 0.015 to 1.5. the product should not be fortified with synthetic tryptophan or peptides.

Endogenous inorganic phosphate is an important regulator of pathways. In addition it is important to avoid deficiencies. When phosphate is included in the product, the amount per daily dose will be in the range 20–2000 mg, and preferably 100–1000 mg.

Citrate can be used in the form of pure cristalline citric acid or salts thereof, but also be present in extracts of fruit (oranges, lime). When citrate is included the pH of the final product should be in the range 3–8 and preferably 6–7.5 The amount of citrate per daily dose of product should be 0.1–6 g and preferably 0.3–3 g, dependant on the use of the product.

Orotate is defined as being those components that after consumptions that after consumption will provide orotate ions in the blood plasma. Suitable sources are orotic acid (6-carboxy-2,4-dihydroxypyrimidine), salts thereof such as sodium or potassium or zinc salts, esters such as choline—or methyl esters and extracts that are rich in orotic acid such as certain extracts from liver. Also precursors like arginine, glutamine or aspartate can be used.

In order to be effective, the product should comprise 0.1–8 g orotate per daily dose. This amount is in general less than

the amount of ribose or glutamine in the product and should be determined by using the criterium that about 0.04–0.3 g orotate is given per kg body weight. When arginine or aspartate is used as precursor for orotate, per day more than 1 g should be administered and preferably more than 5 grams. Orotate is claimed to ensure proper biosynthesis of pyrimidines and to neutralise excess of ribose that may get formed in the specific conditions of that patient. This also would ensure sufficient tissue levels of beta-alamine, carnosine and anserine.

It is found that inclusion of vitamin B12 in the product increases nucleotide levels in tissue. As source of vitamin B12, cobalamines, such as cyanocobalamine can be used. The quantity should be 0.2–4000 μg and preferably 0.5–5 μg per daily doses.

As mentioned above it is important to include vitamin B6 when the product is meant to be used for the prevention or treatment of some cerebral disorders or neurodegenerative diseases such as Parkinson, Huntington, Alzheimer, ADHD, depression, schizophrenia and mood disorders. This amount should be 2–10 mg and preferably 2–4 mg per daily dose. For other applications, such as infant formula, the product can comprise less vitamin B6. In general the preparations according to the invention will comprise 0.3–10 and preferably 0.5–4 mg per daily dose.

Selenium is preferably included into the product to ensure a proper supply of this key component for the enzyme catalase. As selenium source selenium salts such as sodium selenite can be used but also extracts of raw materials that are rich in selenium such as selenium yeast. The amount to be included in 10–300 μg and in particular for products for adults 40–150 μg per daily doses.

Other food grade components such as lipids (in general), additional vitamins, minerals, trace elements, carmitine, creatine, coenzym Q10, conjugated linoleic acid (CLA), alfa-lipoic acid or their functional analogues or fibre could be part of the composition, dependant on the application of the nutritional compositions. The use of these components for disease-specific nutritional compositions is disclosed in the prior art.

Long-chain polyunsaturated fatty acids, especially ω-3 unsaturated fatty acids such as DHA, EPA and stearidonic acid are preferably included in compositions intended for enhancing the immune function of patients. These fatty acids may originate from known sources such as marine algal oils, fish oils, selected vegetable oils (e.g. from Echium) or from synthetic sources.

The invention also pertains to a method of enhancing uric acid antioxidant capacity in blood plasma, comprising administering an effective amount of at least one compound selected from ribose and folate. The effective amounts depend on the condition of the subject and on the required antioxidant power, and can be selected from those given in table 1. Other antioxidants may also be present, although preferably at lower doses than commonly used.

Clinical use

Conditions for which the compositions of the invention can be used include in general those conditions in which the biosynthetic capacity is insufficient, e.g. due to metabolic disorders or nutrient deficiencies. In such conditions large amounts of specific nucleotides and/or related metabolites are required locally, e.g., due to diseases or local disorders or specific demands, and transport of nutrients and TNM components is imparted.

Persons that would benefit from these products are persons that have a general insufficiency to synthesize, salvage or convert nucleotides such as those suffering from protein-energy malnutrition and person that suffer from an inherited disturbance in the expression of one of the enzymes that are involved in TNM. The same general problem can occur in premature or young infants due to an under development of their enzyme systems. Also many elderly persons and persons suffering from liver problems (for example due to alcohol abuse or hepatitis) have insufficient capacity for nucleotide synthesis due to imparted enzyme systems and need support of the most critical steps in TNM.

The product is also meant to be used in those conditions that locally and sometimes temporarily very high requirements exist for nucleotides or related metabolites and the requirements cannot readily be met by available biochemical capacity. In case of trauma (for example after an accident or surgery) locally high amounts of all nucleotides must be generated in order to repair (replace) damaged tissue. During various inflammatory disorders during some stage lymphocytes rapidly proliferate, which puts high demands on nucleotide supply to synthesizing tissues.

In addition it is beneficial to increase total antioxidant capacity of the relevant extracellular fluids, especially in those situations that an uncontrolled inflammatory reaction exits.

High amounts of nucleotides must also be available locally during spermatogenesis, during lactation and in bone marrow during periods of (increased) synthesis of blood components. The product is therefore also claimed to be useful for improving subfertility, increasing milk quantity and quality of milk during lactation and useful when used after surgery. Nutritional products according for these types of patients may be fortified with trace elements, minerals and vitamins to meet their nutritional requirements. Products for use after surgery should preferably comprise a protein and lipid source isolated from egg or milk and growth factors, such as insulin-like growth factors and epidermal growth factors.

Under normal conditions gut epithelial cells have a relatively short half-life and are produced from specialized stem cells. During proliferation but also during maturation (migration) nucleotide and/or energy requirements of these cells are high. Several gut disorders are associated with a very high throughput of gut epithelial cells, which requires local availability of large amounts of various nucleotides. Inclusion of glutamine or its equivalents will further enhance the effectivity of the nutritional compositions of the invention, especially when administered at more than 1 g per daily dosage.

Also during recovery after chemo- or radiotherapy, large amounts of nucleotides must be available in the right relative proportions. During therapy large members of cells will die due to apoptosis and large amounts of nucleotides are released. Once these have been metabolized extra biosynthetic capacity is required, to meet the high demands. The nutritional compositions of the invention will support recovery after damage due to chemotherapy especially in the mucosal tissue.

Temporary insufficiencies in biosynthetic capacity of nucleotides become in particular evident during ischaemic situations as may occur during trauma, surgery and cardio- and cerebro-vascular problems. The decreased blood supply causes that low amounts of nucleotides or related metabolites are offered to the underlying tissues. It is thought that by supplying the claimed product, the concentrations of the required nucleotides or related products are sufficiently higher to improve the status of tissue that suffers from a bad

blood supply. In case the tissue is brain tissue, the product will decrease the amount of excitatory amino acids, such as glutamate, that will be released.

Also during and after heavy exercise, existing deficiencies in tissue nucleotide content and/or imparted capacity for TNM become evident. Pools of ATP will rapidly become depleted and it will take a relatively long time before tissue that has been damaged during exercise will be repaired or replaced. This will lead to muscle soreness and injuries. The product is also claimed to be useful in treatment of MS and fatigue.

Nucleotides play an important regulating role in cell metabolism, and can serve as donor or acceptor of amine groups in catabolism of asparatate in periods that demands on muscle are high and blood supply does not meet requirements.

The product is further claimed to be useful in the prevention of cancer. Without wanting to be bound by theory it is thought that adenine and PRPP are involved in the polymerization of ADP-ribose onto nuclear proteins, which are important for cell regulation and DNA repair. Improvement of the possibilities for repair of damaged DNA then prevents the development of tumor cells.

The product is further claimed to be especially useful in the prevention and treatment of rheumatoid arthritis and other joint problems. It is thought that the product operates by means of its effect on sulphation capacity. This would lead to an increase in concentrations and/or variety of sulphated polysaccharides in interstitial and synovial fluid, thus improving the lubricating properties.

It is also claimed that the products improve immune function. Without wanting to be bound by theory, we think that this effect is thought to the effect of the formulations on mucus composition and levels of endogenous amounts of glycoproteins, immunoglobulins and proteoglycans. These components are essential for proper functioning of membrane structures including their recognition function and the functioning of enzymes, in particular with regard to their specificity. This beneficial effect on the barrier function of mucus is claimed to be particularly relevant for persons suffering from respiratory infections or common cold.

The preparations are further claimed to be useful in preventing problems with blood supply to tissues. This is thought to be due to the improvement of blood clotting parameters and the effect on microvascularisation. The products are further helpful in the treatment of cystic fibroses and treatment or prevention of malaria and tuberculose. The effect of the product is further enhanced by fortification with vitamin B6, especially in an mount of more than 3 mg/day.

The product is further claimed to be useful in the treatment of intoxications, e.g. due to drug abuse but also due to problems with bilirubine metabolism. It is thought that the product ensures sufficient glucuronidation or sulphation conjugation. The product also supports regulation of steroid metabolism.

The product is also claimed to be useful in the treatment of pain as may become evident in attacks of migraine and pain on the chest after an infarct. It is thought that the product regulates the amount of adenosine in such a way that the vasodilatation effect is maintained and local sudden rises in concentration are avoided.

The product is also claimed to be helpful in treating patients suffering from or at risk for developing renal failure, ischaemic events such as infarcts and cerebrovascular accidents, inflammatory bowel disease and cancer. This is thought to occur via restoration of tissue levels of beta-alanine, carnosine and anserine.

In case the product is used in the treatment or prevention of cardio or cerebro vascular problems it is useful to include creatine, carmitine, coenzym Q10, tannine, vit B12, vit B6, Zn and/or Mg in the product.

The product is also claimed to be beneficial in the treatment of disorders associated with imbalances in neurotransmitter levels in the brain. Imbalances in neurotransmitter levels in the brain occur in persons that suffer from Parkinson's disease, Alzheimer's disease, ADHD, schizophrenia, depression and mood disorders. The effect is thought to get obtained via an increase in effectivity of vitamin B6, either co-administered or already present in the body, and a regulation of metabolism of dopamine, adrenaline, serotonin and noradrenaline.

TABLE 1

List of the active components of the nutritional preparation:

Ingredient/	Amount per daily dose			
component	full range	preferred range	most preferred range	
Ribose	0.5–40	1–15	2–10	g
Folate	>100	250 μg–20 mg	400 μg–5 mg	
Niacin		>4	30–200	niacin equivalents
Magnesium		>20	100–500	mg
Glucose		1–50	2–20	g
Protein		0–120 g		
Histidine		0.2–5	0.3–3	g
Inorganic phosphate		20–2000	100–1000	mg
Vitamin B1		0.4–10	1–8	mg
Vitamin B12		0.2–4000	0.5–5	μg
Glutamine		0.5–30	1–10	g
Orotate		0.1–8 g		
Citrate		0.1–6	0.3–3	g
Selenium		10–300	40–150	μg
Vitamin B6		0.3–10	0.5–4	mg

EXAMPLES

1. Nutritional preparation for improving exercise performance of sportsman Ingredients per daily dose:
   - 3 g of an amino acid mixture that consists of 10 wt % histidine, 10 wt % isoleucine, 20 wt % leucine, 15 wt % lysine, 12 wt % methionine, 10 wt % phenylalanine and 13 wt % threonine
   - 5 g D-ribose
   - 10 g maltodextrins DH 19
   - 2 g MCT oil
   - 200 μg Folic acid
   - 30 mg Niacin
   - 0.5 g orotic acid
   - 500 mg magnesium phosphate
   - 50 mg α-lipoic acid

The product is packed in a sachet for reconstitution in a glass of water, fruit juice or milk, or similar liquid.

2. Powdered nutritional supplement that contains per daily dose:
   - 17 g of an amino acid mixture that consists of 2 g glutamine, 0.7 g arginine, 0.5 g histidine, 0.4 g isoleucine, 1.0 g leucine, 0.9 g lysine, 0.3 g methionine, 0.3 g phenylalanine, 0.3 g threonine, 0.6 g valine.
   - 6 g D(−)ribose
   - 20 g maltodextrin
   - 300 μg folic acid
   - 40 mg niacin

2 mg thiamin HCl
2 μg cyanocobalamine
1.0 g sodium orotate
400 mg alfa-lipoic acid
225 μg biotin
15 mg pantothenic acid
1 mg pyridoxineoHCl
0.5 g potassium carbonate
0.5 g sodium citrate
0.5 g sodium chloride
0.6 g magnesium chloride
0.6 g tri-calcium phosphate
50 mg ascorbic acid
20 mg α-tocophenol
Trace of flavoring agent

Eight daily doses of are packed in a 500 g can.

3. Dietetic preparation for gastrointestinal problems (IBD, M. Crohn, sepsis, food allergy)

Oral rehydration drink that contains per liter
5 g yeast extract
3 g D(−)ribose
5 g maltodextrins DH 10
600 μg folic acid
5 μg cyanocobalamine
20 mg zinc sulfate
4 mg Cu chloride
300 mg magnesium phosphate
1 g orotic acid
5 g glutamyl-glutamast
160 μg sodium selenite

3. Pharmaceutical preparation for pregnant or lactating women

Cup having a volume of 200 ml that comprises a pudding based on skimmed milk powder that comprises per 200 ml:
3 g D-ribose
400 μg folic acid
4 μg cyanocobalamine
4 mg pyridoxamine
100 mg magnesium chloride
25 mg zinc citrate
0.4 g methionine
10 g maltodextrin
1 g pectin

4. Supplement for persons suffering from vascular problems (cardiovascular, atheroscleroses, cerebrovascular diseases, infarcts, decreased blood supply to legs, bad vessel quality) Bar (weight about 28 g ingredients, including moisture) that comprises:
15 g glucose syrup
4 g D-ribose
400 μg folic acid
3 μg cyanocobalamine
30 mg zinc sulfate
250 mg magnesium carbonate
3 mg pyridoxamine
2 g arginine HCl
2 g dried fruit (apple, pear and apricot)
2 g grain flocks (rye, wheat, oats)
5 g mixture of nut pieces (hazelnut)
1 g creatine
30 mg alfa tocophenol
50 mg ascorbic acid
20 mg coenzym Q10

5. Product to support liver function in case of liver cirrhoses, alcohol abuse, hepatitis, etc.
5 g Yeast extract
3 g D(−)ribose
2 g glutamine
10 g maltodextrins DH 19
400 μg folic acid
4 ug cyanocobalamine
3 mg pyridoxine
1 g arginine
2 g sodium orotate
1 g aspartate
2 mg thiamin, HCl
40 mg zinc carbonate
4 mg copper carbonate
150 μg potassium selenate

6. Soup for the elderly

A dried soup concentrate is prepared from
5 g Powdered broccoli extract, providing 180 μg folate
5 g Powdered yeast extract, providing 100 μg folate and 3 g ribose
200 ug folic acid
1 g D(−) ribose
3 μg cyanocobalamine
0.5 g sodium chloride
0.4 g potassium chloride
1 g dried carrot pieces
1 g dried onion pieces
20 mg zinc sulfate
2 g maltodextrin

7. Complete formula for prematures to reduce risk of jaundice

Infant formula for reducing risk of jaundice comprising per 100 g powder;
12.4 g protein equivalents in the form of whey/casein 60/40 w/w
54.8 g digestable carbohydrates of which 2 g D(−) ribose
21.1 g lipids
5 g fibre

standard quantities of trace elements, minerals and vitamins as known in the art except that 110 ug folic acid is included.

8. Supplement for improving immune function

Cookie for improving immune function

A Wheat-based cookie comprising per 30 g
2 g glutamine
3 g ribose
10 g maltodextrin that includes 200 ug docosabexaenoic acid, 200 ug Eicosapentaenoic acid and 100 ug arachidonic acid
20 mg zinc
100 mg magnesium
400 ug folic acid
3 ug vitamin B12

4 mg copper

0.3 g citric acid

9. Powder for prevention and treatment of osteoarthritis, comprising per 40 g

300 ug folic acid

4 g D(−) ribose

0.5 g methionine

2 ug cyanocobalamine

0.8 g sodium citrate

0.5 g histidine

4 mg pyridoxine HCl

14 mg zinc

200 mg magnesium

200 mg sulphate

9 g casein

20 g maltodextrines

4 g soy lecithin

We claim:

1. A nutritional composition, comprising:

ribose and folate in amounts which are effective to support total nucleotide metabolism, and

0.1–8 g orotate per 5 g of ribose.

2. The nutritional composition of claim **1**, wherein said nutritional composition is in a unit dosage form, said ribose is an amount of 0.5 g–40 g per unit dosage, and said nutritional composition further comprises 100 μg–20 mg of folate per 1 g–15 g of free ribose.

3. The nutritional composition of claim **2**, wherein said nutritional composition is in a unit dosage form, said ribose is an amount of 0.5 g–40 g per unit dosage, and said nutritional composition further comprises 250 μg–5 mg of folate per 1 g–15 g of free ribose.

4. The nutritional composition of claim **1**, further comprising at least one component selected from the group consisting of magnesium, niacin, selenium, thiamine, glucose, citrate, histidine, phosphate, sulfate and vitamin B12.

5. The nutritional composition of claim **4**, wherein said nutritional composition is in a unit dosage form, and further comprises a 4–200 mg niacin equivalents per 0.5 g–40 g unit dosage of ribose or per 5 g of the ribose.

6. The nutritional composition of claim **4**, further comprising 1–50 g glucose per said unit dosage of ribose or per 5 g of ribose.

7. The nutritional composition of claim **1**, wherein said nutritional composition is in a unit dosage form, and further comprises 5–120 g of protein per 0.5–40 g unit dosage of ribose or per 5 g of ribose.

8. The nutritional composition of claim **1**, wherein said nutritional composition is in a unit dosage form, and further comprising more than 0.3 g of histidine per 0.5–40 g unit dosage of ribose or per 5 g of ribose.

9. The nutritional composition of claim **1**, wherein said nutritional composition is in a unit dosage form, and further comprising, per 0.5–40 g unit dosage of ribose or per 5 g of ribose, more than 0.5 g of glutamine, and/or more than 1 g of aspartate and/or more than 1 g of arginine.

10. The nutritional composition of claim **1**, wherein said nutritional composition is in a unit dosage form, and further comprising, per 0.5.–40 g unit dosage of ribose or per 5 g of ribose, more than 2 g of a mixture of amino acids or amino acid equivalents containing 3–10 wt % histidine, 5–15 wt % methionine, 5–15 wt % phenylalanine and 5–15 wt % threonine.

11. The nutritional composition of claim **10**, wherein said histidine, glutamine, aspartate and/or arginine being present for at least 50% in the form of free amino acids or dipeptides.

12. The nutritional composition of claim **1**, wherein said nutritional composition is in a dry form and in an amount effective for the treatment of a conduction selected from trauma, surgery, inflammation (either acute and chronic), subfertility, lactation problems, got disorders, infant nutrition (jaundice, gut maturation), cancer, arthritis (osteo-, (rheumatoid) and other joint problems, vascular problems and cardio-or cerebro vascular problems, ischaemia (imparted peripheral blood supply, infarcts), ageing, impaired immune function (such as after chemotherapy and during AIDS), burns, sepsis, malnutrition, problems with liver or kidneys, malaria, cystic fibrosis, tuberculose, fatigue, MS, migraine, neurological problems such as Huntington, Parkinson, Alzheimer, schizophrenia and depression, respiratory infections, improvement of sports results, muscle soreness, drug intoxication and pain.

13. The nutritional composition of claim **1**, being in the form of a food supplement.

14. The nutritional composition of claim **1**, wherein said nutritional composition is in a unit dosage form, and further comprising Ω3 polyunsaturated fatty acids at a level of more then 500 mg, per 0.5–40 g unit dosage of ribose or per 5 g of ribose, for the improvement of immune functions.

15. The nutritional composition of claim **1**, wherein said nutritional composition is in a unit dosage form, and further comprising glutamine at a level of 1–10 g per 0.5–40 g unit dosage of ribose or per 5 g of ribose, for the improvement of immune functions.

16. The nutritional composition of claim **1**, wherein said nutritional composition is in a unit dosage form, and further comprising vitamin B6 at a level of 0.3–10 mg per 0.5–40 g unit dosage of ribose or per 5 g of ribose, for the improvement of malaria or neurologic disorders.

17. The nutritional composition of claim **1**, further comprising at least one component selected from the group consisting of carnitine, creatine, coenzyme Q10, taurine, betaine, alpha-lipoic acid, vitamin B6, vitamin B12, zinc and magnesium for the prevention or treatment of cardiovascular or cerebrovascular disorders.

18. The nutritional composition of claim **1**, wherein said nutritional composition is in a liquid form and in an amount effective for the treatment of a condition selected from trauma, surgery, inflammation (either acute and chronic), subfertility, lactation problems, gut disorders, infant nutrition (jaundice, gut maturation), cancer, arthritis (osteo-, rheumatoid) and other joint problems, vascular problems and cardio-or cerebro vascular problems, ischaemia (imparted peripheral blood supply, infarcts), ageing, impaired immune function (such as after chemotherapy and during AIDS), burns, sepsis, malnutrition, problems with liver or kidneys, malaria, cystic fibrosis, tuberculose, fatigue, MS, migraine, neurological problems such as Huntington, Parkinson, Alzheimer, schizophrenia and depression, respiratory infections, improvement of sports results, muscle soreness, drug intoxication and pain.

19. A nutritional composition, comprising:

ribose and folate in amounts which are effective to support total nucleotide metabolism, and

more than 500 mg of Ω-3 polyunsaturated fatty acids per 5 g of ribose.

20. A nutritional composition, comprising:

ribose and folate in amounts which are effective to support total nucleotide metabolism, and

between 0.1 to 6 g citrate per 0.5–40 g unit dosage of ribose or per 5 g of ribose.

21. A nutritional composition according to claim **20**, comprising between 0.3 and 3 g citrate per said unit dosage of ribose or per 5 g of ribose.

22. A nutritional composition according to claim **20**, comprising between 1 and 50 g glucose per said unit dosage of ribose or per 5 g of ribose.

* * * * *

EXHIBIT G

US006953679B2

(12) **United States Patent**
Salvati et al.

(10) **Patent No.: US 6,953,679 B2**
(45) **Date of Patent: Oct. 11, 2005**

(54) **METHOD FOR THE PREPARATION OF FUSED HETEROCYCLIC SUCCINIMIDE COMPOUNDS AND ANALOGS THEREOF**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 183 days.

(21) Appl. No.: **10/024,878**

(22) Filed: **Dec. 19, 2001**

(65) **Prior Publication Data**

US 2005/0119228 A1 Jun. 2, 2005

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/885,381, filed on Jun. 20, 2001.

(60) Provisional application No. 60/284,730, filed on Apr. 18, 2001, and provisional application No. 60/233,519, filed on Sep. 19, 2000.

(51) **Int. Cl.**[7] **C12P 17/10**; C12N 1/00

(52) **U.S. Cl.** **435/121**

(58) **Field of Search** 435/121, 183, 435/243, 253.5, 254.1, 886

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(57) **ABSTRACT**

Fused cyclic compounds, methods of using such compounds in the treatment of nuclear hormone receptor-associated conditions such as cancer and immune disorders, and pharmaceutical compositions containing such compounds.

4 Claims, No Drawings

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METHOD FOR THE PREPARATION OF FUSED HETEROCYCLIC SUCCINIMIDE COMPOUNDS AND ANALOGS THEREOF

This application is a continuation-in-part of U.S. application Ser. No. 09/885,381, filed Jun. 20, 2001, now pending which claims the benefit of U.S. Provisional Application Nos. 60/233,519, filed Sep. 19, 2000, and 60/284,730, filed Apr. 18, 2001. The entire disclosure of each of these applications is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to fused cyclic compounds, to methods of using such compounds in the treatment of nuclear hormone receptor-associated conditions such as cancer, and to pharmaceutical compositions containing such compounds.

BACKGROUND OF THE INVENTION

Nuclear hormone receptors (NHR's) constitute a large super-family of ligand-dependent and sequence-specific transcription factors. Members of this family influence transcription either directly, through specific binding to the promoter target genes (Evans, in *Science* 240: 889–895 (1988)), or indirectly, via protein-protein interactions with other transcription factors (Jonat et al., *Cell* 62: 1189–1204 (1990), Schuele et al., *Cell* 62: 1217–1226 (1990), and Yang-Yen et al., *Cell* 62: 1205–1215 (1990)). The nuclear hormone receptor super-family (also known in the art as the "steroid/thyroid hormone receptor super-family") includes receptors for a variety of hydrophobic ligands, including cortisol, aldosterone, estrogen, progesterone, testosterone, vitamine D3, thyroid hormone and retinoic acid (Evans, 1988, supra). In addition to these conventional nuclear hormone receptors, the super-family contains a number of proteins that have no known ligands, termed orphan nuclear hormone receptors (Mangelsdorf et al., *Cell* 83: 835–839 (1995), O'Malley et al., *Mol. Endocrinol.* 10: 1293 (1996), Enmark et al., *Mol. Endocrinol.* 10, 1293–1307 (1996) and Giguere, *Endocrin. Rev.* 20, 689–725 (1999)). The conventional nuclear hormone receptors are generally transactivators in the presence of ligand, and can either be active repressors or transcriptionally inert in the absence of ligand. Some of the orphan receptors behave as if they are transcriptionally inert in the absence of ligand. Others, however, behave as either constitutive activators or repressors. These orphan nuclear hormone receptors are either under the control of ubiquitous ligands that have not been identified, or do not need to bind ligand to exert these activities.

In common with other transcription factors, the nuclear hormone receptors have a modular structure, being comprised of three distinct domains: an N-terminal domain of variable size containing a transcriptional activation function AF-1, a highly conserved DNA binding domain and a moderately conserved ligand-binding domain. The ligand-binding domain is not only responsible for binding the specific ligand but also contains a transcriptional activation function called AF-2 and a dimerisation domain (Wurtz et al., *Nature Struc. Biol.* 3, 87–94 (1996), Parker et al., *Nature Struc. Biol.* 3, 113–115 (1996) and Kumar et al., *Steroids* 64, 310–319 (1999)). Although the overall protein sequence of these receptors can vary significantly, all share both a common structural arrangement indicative of divergence from an ancestral archetype, and substantial homology (especially, sequence identity) at the ligand-binding domain.

The steroid binding nuclear hormone receptors (SB-NHR's) comprise a sub-family of nuclear hormone receptors. These receptors are related in that they share a stronger sequence homology to one another, particularly in the ligand binding domain (LBD), than to the other members of the NHR super-family (Evans, 1988, supra) and they all utilize steroid based ligands. Some examples of this sub-family of NHR's are the androgen receptor (AR), the estrogen receptor (ER), the progesterone receptor (PR), the glucbcorticoid receptor (GR), the mineralocorticoid receptor (MR), the aldosterone receptor (ALDR) and the steroid and xenobiotic receptor (SXR) (Evans et al., WO 99/35246). Based on the strong sequence homology in the LBD, several orphan receptors may also be members of the SB-NHR sub-family.

Consistent with the high sequence homology found in the LBD for each of the SB-NHR's, the natural ligands for each is derived from a common steroid core. Examples of some of the steroid based ligands utilized by members of the SB-NHR's include cortisol, aldosterone, estrogen, progesterone, testosterone and dihydrotestosterone. Specificity of a particular steroid based ligand for one SB-NHR versus another is obtained by differential substitution about the steroid core. High affinity binding to a particular SB-NHR, coupled with high level specificity for that particular SB-NHR, can be achieved with only minor structural changes about the steroid core (e.g., Waller et al., *Toxicol. Appl. Pharmacol.* 137, 219–227 (1996) and Mekenyan et al., *Environ. Sci. Technol.* 31, 3702–3711 (1997), binding affinity for progesterone towards the androgen receptor as compared to testosterone).

Numerous synthetically derived steroidal and non-steroidal agonists and antagonists have been described for the members of the SB-NHR family. Many of these agonist and antagonist ligands are used clinically in man to treat a variety of medical conditions. RU486 is an example of a synthetic agonist of the PR, which is utilized as a birth control agent (Vegeto et al., *Cell* 69: 703–713 (1992)), and Flutamide is an example of an antagonist of the AR, which is utilized for the treatment of prostate cancer (Neri et al, *Endo.* 91, 427–437 (1972)). Tamoxifen is an example of a tissues specific modulator of the ER function, that is used in the treatment of breast cancer (Smigel, *J. Natl. Cancer Inst.* 90, 647–648 (1998)). Tamoxifen can function as an antagonist of the ER in breast tissue while acting as an agonist of the ER in bone (Grese et al., *Proc. Natl. Acad. Sci. USA* 94, 14105–14110 (1997)). Because of the tissue selective effects seen for Tamoxifen, this agent and agents like it are referred to as "partial-agonist" or partial-antagonist". In addition to synthetically derived non-endogenous ligands, non-endogenous ligands for NHR's can be obtained from food sources (Regal et al., *Proc. Soc. Exp. Biol. Med.* 223, 372–378 (2000) and Hempstock et al., *J. Med. Food* 2, 267–269 (1999)). The flavanoid phytoestrogens are an example of an unnatural ligand for SB-NHR's that are readily obtained from a food source such as soy (Quella et al., *J. Clin. Oncol.* 18, 1068–1074 (2000) and Banz et al., *J. Med. Food* 2, 271–273 (1999)). The ability to modulate the transcriptional activity of individual NHR by the addition of a small molecule ligand, makes them ideal targets for the development of pharmaceutical agents for a variety of disease states.

As mentioned above, non-natural ligands can be synthetically engineered to serve as modulators of the function of NHR's. In the case of SB-NHR's, engineering of an unnatural ligand can include the identification of a core structure which mimics the natural steroid core system. This can be achieved by random screening against several SB-NHR's or

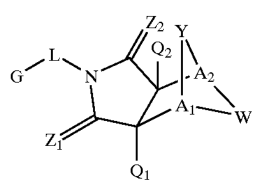
through directed approaches using the available crystal structures of a variety of NHR ligand binding domains (Bourguet et al., *Nature* 375, 377–382 (1995), Brzozowski, et al., *Nature* 389, 753–758 (1997), Shiau et al., *Cell* 95, 927–937 (1998) and Tanenbaum et al., *Proc. Natl. Acad. Sci. USA* 95, 5998–6003 (1998)). Differential substitution about such a steroid mimic core can provide agents with selectivity for one receptor versus another. In addition, such modifications can be employed to obtain agents with agonist or antagonist activity for a particular SB-NHR. Differential substitution about the steroid mimic core can result in the formation of a series of high affinity agonists and antagonists with specificity for, for example, ER versus PR versus AR versus GR versus MR. Such an approach of differential substitution has been reported, for example, for quinoline based modulators of steroid NHR in *J. Med. Chem.,* 41, 623 (1999); WO 9749709; U.S. Pat. No. 5,696,133; U.S. Pat. No. 5,696,130; U.S. Pat. No. 5,696,127; U.S. Pat. No. 5,693,647; U.S. Pat. No. 5,693,646; U.S. Pat. No. 5,688,810; U.S. Pat. No. 5,688,808 and WO 9619458, all incorporated herein by reference.

The compounds of the present invention comprise a core which serves as a steroid mimic, and are useful as modulators of the function of steroid binding nuclear hormone receptors, as well as other NHR as described following.

SUMMARY OF THE INVENTION

The present invention provides fused cyclic compounds of the following formula I and salts thereof, which compounds are especially useful as modulators of nuclear hormone receptor function:

(I)



As used in formula I, and throughout the specification, the symbols have the following meanings unless otherwise indicated, and are, for each occurrence, independently selected:

G is an aryl or heterocyclo (e.g., heteroaryl) group, where said group is mono- or polycyclic, and which is optionally substituted at one or more positions, preferably with hydrogen, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, halo, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, aryl or substituted aryl, heterocyclo or substituted heterocyclo; arylalkyl or substituted arylalkyl, heterocycloalkyl or substituted heterocycloalkyl, CN, $R^1OC{=}O$, $R^1C{=}O$, $R^1C{=}S$, $R^1HNC{=}O$, $R^1R^2NC{=}O$, $HOCR^3R^{3'}$, nitro, R^1OCH_2, R^1O, NH_2, NR^4R^5, SR^1, $S{=}OR^1$, SO_2R^1, SO_2OR^1, $SO_2NR^1R^{1'}$, $(R^1O)(R^{1'}O)P{=}O$, oxo, $(R^1)(R^{1'})P{=}O$, or $(R^{1'})(NHR^1)P{=}O$;

Z_1 is O, S, NH, or NR^6

Z_2 is O, S, NH, or NR^6

A_1 is CR^7 or N;

A_2 is CR^7 or N;

Y is J—J'—J" where J is $(CR^7R^{7'})n$ and n=0–3, J' is a bond or O, S, S═O, SO_2, NH, NR^7, C═O, OC═O, $NR^1C{=}O$, $CR^7R^{7'}$, $C{=}CR^8R^{8'}$, $R^2P{=}O$, $R^2P{=}S$, $R^2OP{=}O$, $R^2NHP{=}O$, $OP{=}OOR^2$, $OP{=}ONHR^2$, $OP{=}OR^2$, OSO_2, $C{=}NR^7$, NHNH, $NHNR^6$, NR^6NH, N═N, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo or aryl or substituted aryl, and J" is $(CR^7R^{7'})n$ and n=0–3, where Y is not a bond (i.e., if J' is a bond, then in at least one of J or J" (each defined as $(CR^7R^{7'})n$), n is not zero);

W is $CR^7R^{7'}$—$CR^7R^{7'}$, $CR^8{=}CR^{8'}$, $CR^7R^{7'}$—C═O, NR^9—$CR^7R^{7'}$, $N{=}CR^8$, N═N, NR^9–$NR^{9'}$, S—$CR^4R^{7'}$, SO—$CR^7R^{7'}$, $SO_2CR^7R^{7'}$, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, or aryl or substituted aryl, wherein when W is not NR^9—$CR^7R^{7'}$, $N{=}CR^8$, N═N, NR^9—$NR^{9'}$, S—$CR^7R^{7'}$, SO—$CR^7R^{7'}$, SO_2—$CR^7R^{7'}$, or heterocyclo or substituted heterocyclo, then J' must be O, S, S═O, SO_2, NH, NR^7, OC═O, $NR^1C{=}O$, $OP{=}OOR^2$, $OP{=}ONHR^2$, OSO_2, NHNH, $NHNR^6$, NR^6NH, or N═N;

Q_1, is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocycloalkyl or substituted heterocycloalkyl, arylalkyl or substituted arylalkyl, alkynyl or substituted alkynyl, aryl or substituted aryl, heterocyclo (e.g., heteroaryl) or substituted heterocyclo (e.g., substituted heteroaryl), halo, CN, $R^1OC{=}O$, $R^4C{=}O$, $R^5R^6NC{=}O$, $HOCR^7R^{7'}$, nitro, R^1OCH_2, R^1O, NH_2, $C{=}OSR^1$, SO_2R^1 or NR^4R^5;

Q_2 is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocycloalkyl or substituted heterocycloalkyl, arylalkyl or substituted arylalkyl, alkynyl or substituted alkynyl, aryl or substituted aryl, heterocyclo (e.g., heteroaryl) or substituted heterocyclo (e.g., substituted heteroaryl), halo, CN, $R^1OC{=}O$, $R^4C{=}O$, $R^5R^6NC{=}O$, $HOCR^7R^{7'}$, nitro, R^1OCH_2, R^1O, NH_2, $C{=}OSR^1$, SO_2R^1 or NR^4R^5;

L is a bond, $(CR^7R^{7'})n$, NH, NR^5, $NH(CR^7R^{7'})n$, or $NR^5(CR^7R^{7'})n$, where n=0–3;

R^1 and $R^{1'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkyalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl;

R^2 is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl;

R^3 and $R^{3'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, halo, CN, hydroxylamine, hydroxamide, alkoxy or substituted alkoxy, amino, NR^1R^2, thiol, alkylthio or substituted alkylthio;

R^4 is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or

substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, $R^1C{=}O$, $R^1NHC{=}O$, SO_2OR^1, or $SO_2NR^1R^{1'}$;

R^5 is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, $R^1C{=}O$, $R^1NHC{=}O$, SO_2R^1, SO_2OR^1, or $SO_2NR^1R^{1'}$;

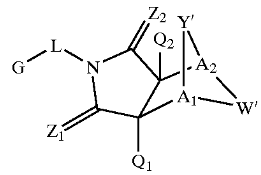
R^6 is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, CN, OH, OR^1, $R^1C{=}O$, $R^1NHC{=}O$, SO_2R^1, SO_2OR^1, or $SO_2NR^1R^{1'}$;

R^7 and $R^{7'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, halo, CN, OR^1, nitro, hydroxylamine, hydroxylamide, amino, NHR^4, NR^2R^5, NOR^1, thiol, alkylthio or substituted alkylthio, $R^1C{=}O$, $R^1(C{=}O)O$, $R^1OC{=}O$, $R^1NHC{=}O$, SO_2R^1, SOR^1, $PO_3R^1R^{1'}$, $R^1R^{1'}$ $NC{=}O$, $C{=}OSR^1$, SO_2OR^1, or $SO_2NR^1R^{1'}$, or, wherein A_1 or A_2 contains a group R^7 and W contains a group R^7, said R^7 groups of A_1 or A_2 and W together form a heterocyclic ring;

R^8 and $R^{8'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkyalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, nitro, halo, CN, OR^1, amino, NHR^4, NR^2R^5, NOR^1, alkylthio or substituted alkylthio, $C{=}OSR^1$, $R^1OC{=}O$, $R^1C{=}O$, $R^1NHC{=}O$, $R^1R^{1'}NC{=}O$, SO_2OR^1, $S{=}OR^1$, SO_2R^1, $PO_3R^1R^{1'}$, or $SO_2NR^1R^{1'}$; and

R^9 and $R^{9'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, CN, OH, OR^1, $R^1C{=}O$, $R^1OC{=}O$, $R^1NHC{=}O$, SO_2R^1, SO_2OR^1, or $SO_2NR^1R^{1'}$.

Compounds within formula I are novel, a preferred subgenus of which is the following formula Ia:



(Ia)

where G, L, Z_1, Z_2, A_1, A_2, Q_1 and Q_2 are as defined above;

Y' is J—J'—J" where J is $(CR^7R^{7'})$n and n=0–3, J' is a bond or O, S, S═O, SO_2, NH, NR^7, $CR^7R^{7'}$, $R^2P{=}O$, $R^2P{=}S$, $R^2OP{=}O$, $R^2NHP{=}O$, $OP{=}OOR^2$, $OP{=}ONHR^2$, OSO_2, NHNH, $NHNR^6$, NR^6NH, N═N, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, or heterocyclo or substituted heterocyclo, and J" is $(CR^7R^{7'})$n and n=0–3, where Y is not a bond; and

W' is $CR^7R^{7'}$—$CR^7R^{7'}$, $CR^7R^{7'}$—C═O, NR^9—$CR^7R^{7'}$, N═CR^8, N═N, NR^9—$NR^{9'}$, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, or aryl or substituted aryl, wherein, when W" is not NR^9—$CR^7R^{7'}$, N═CR^8, N═N, NR^9—$NR^{9'}$, or heterocyclo or substituted heterocyclo, then J' must be O, S, S═O, SO_2, NH, NR^7, $OP{=}OOR^2$, $OP{=}ONHR^2$, OSO_2, NHNH, $NHNR^6$, NR^6NH, or N═N; or alternatively,

Y' is NR^7—$CR^7R^{7'}$ and W' is CR^8═$CR^{8'}$; or, alternatively,

Y' is $CR^7R^{7'}$—C═O and W' is NR^9—$CR^7R^{7'}$;

where R^2, R^6, R^7,$R^{7'}$, R^8, R^9 and $R^{9'}$ are as defined above and with the provisos that

(1) when Y' is —O—, Q_1 and Q_2 are hydrogen, Z_1 and Z_2 are O, W' is —CH_2—CH_2—, and A_1 and A_2 are CH, then G—L is not phenyl, monosubstituted phenyl or phenyl which is substituted with two or more of the following groups: methoxy, halo, NO_2, methyl, CH_3—S—, OH, CO_2H, trifluoromethyl, —C(O)—C_6H_5, NH_2, 4–7-epoxy, hexahydro-1H-isoindole-1,3(2H) dione, or —C(O)—CH_3;

(2) when Y' is —O—, Q_1 and Q_2 are hydrogen, Z_1 and Z_2 are O, W' is CH_2—CH_2, and one of A_1 and A_2 is CH and the other is CR^7, then G—L is not unsubstituted phenyl;

(3) when Y' is —O—, Q_1 and Q_2 are hydrogen, Z_1 and Z_2 are O, W' is CH_2—CH_2, and one of A_1 and A_2 is CH and the other is C—CH_3, then G—L is not phenyl substituted with chloro and/or methyl;

(4) when Y' is —O— or —S—, Q_1 and Q_2 are hydrogen, Z_1 and Z_2 are O, W' is CH_2—CH_2, and one of A_1 and A_2 is CH and the other is CH or C-alkyl, then G—L is not N-substituted piperazine-alkyl- or N-substituted imidazolidine-alkyl-;

(5) when Y' is —O—; Q_1 and Q_2 are hydrogen, Z_1 and Z_2 are O, W' is CH_2—CH_2, and A_1 and A_2 are CH, then G—L is not oxazole or triazole;

(6) when Y' is —O—; Q_1 and Q_2 are hydrogen or methyl, Z_1 and Z_2 are O, W' is CH_2—CH_2, and A_1 and A_2 are CH or C—CH_3, then G—L is not thiazole or substituted thiazole (in addition such compounds where G—L is optionally substituted thiadiazole or partially saturated thiazole are optionally removed by proviso where A_1 and A_2 are both CH);

(7) when Y' contains a group J' selected from S, S═O, SO_2, NH, NR^7, $R^2P{=}O$, $R^2P{=}S$, $R^2OP{=}O$, $R^2NHP{=}O$, $OP{=}OOR^2$, $OP{=}ONHR^2$, OSO_2, NHNH, NHR^6, NR^6NH or N═N, W' is $CR^7R^{7'}$—

$CR^7R^{7'}$, and Z_1 and Z_2 are O, then G—L is not unsubstituted phenyl;

(8) when Y' is NR^7, W' is unsubstituted or substituted phenyl, and Q_1 and Q_2 are hydrogen, then Z_1, and Z_2 are not O;

(9) when Y' is —O—, Q_1 and Q_2 are hydrogen, Z_1, and Z_2 are O, W' is dihydroisoxazole bearing an optionally substituted phenyl group, and A_1 and A_2 are CH, then G—L is not unsubstituted phenyl or dichlorophenyl;

(10) when Y' is O, Q_1 and Q_2 are hydrogen, Z_1 and Z_2 are O, W' is ethylene oxide, and A_1 and A_2 are CH, then G—L is not methylphenyl or chlorophenyl;

(11) when Y' is $NR^7—CR^7R^{7'}$, W' is $CR^8═CR^{8'}$ Q_1 and Q_2 are hydrogen, A_1 and A_2 are CH, $C—CH_3$, $C—CH_2—C_6H_5$ or $C—CH_2—CH_3$, and Z_1 and Z_2 are O, then G—L is not unsubstituted phenyl, monosubstituted phenyl or methylpyridinyl;

(12) when Y' is $CR^7R^{7'}—C═O$, W' is $NR^9—CR^7R^{7'}$, Q_1 and Q_2 are hydrogen, A_1 and A_2 are CH, and Z_1 and Z_2 are O, then G—L is not unsubstituted phenyl;

(13) when Y' is $CHR^{7'}—NR^7$ where $R^{7'}$ is unsubstituted phenyl, methoxy or ethoxy and R^7 is unsubstituted phenyl, methyl or $—C(O)—C_6H_5$, W' is dimethoxyphenylene or unsubstituted phenylene, Z_1 and Z_2 are O, Q_1 and Q_2 are hydrogen, and A_1 and A_2 are CH, C—CN, $C—C(O)—C_6H_5$, or —C(O)-dimethoxyphenyl, then G—L is not unsubstituted phenyl;

(14) the compound of formula Ia is not 6,10-epithio-4H-thieno-[3',4':5,6]cyclooct[1,2-f]isoindole-7,9(5H,8H)-dione, 8-(3,5-dichlorophenyl)-6,6a,9a, 10,11,12,-hexahydro-1,3,6,10-tetramethyl-2,2,13-trioxide, (6R, 6aR,9aS,10S);

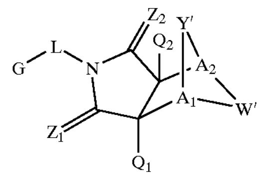
(15) when Y' is O, W' is $—CH_2—CH_2—$, Q_1 and Q_2 are methyl, Z_1 and Z_2 are O, and A_1 and A_2 are CH, then G—L is not unsubstituted phenyl, phenyl substituted with methoxy, phenyl-alkyl-, or morpholine-alkyl, nor is the compound bridged to itself through a group L which is alkylene to form a bis compound;

(16) when Y' is —O—, Q_1 and Q_2 are hydrogen, Z_1 and Z_2 are O, W' is $CR^7R^{7'}—CR^7R^{7'}$; and A_1 and A_2 are CH, then G—L is not an unsubstituted phenyl group; and

(17) when Y' is —O—, Q_1 and Q_2 are hydrogen, Z_1 and Z_2 are O, W' is cyclopentyl, cyclohexyl, 3-phenyl-2-isoxazoline or $CR^7R^{7'}—CR^7R^{7'}$ where R^7 and $R^{7'}$ are each independently defined as Cl, Br, H and 4-butyrolactone and R^7 and $R^{7'}$ are not all simultaneously H, and A_1 and A_2 are CH, then G—L is not an unsubstituted naphthyl ring or a monosubstituted phenyl ring, where said substituent is methoxy, Br, Cl, NO_2, methyl, ethyl, CH_2-phenyl, S-phenyl, or O-phenyl.

Preferably, compounds of formula I are monomeric, and are not comprised within other oligomers or polymers.

Another preferred novel subgenus is that of the following formula Ib:



(Ib)

where G, Z_1, Z_2, Q_1 and Q_2 are as defined above;

Y' is J—J'—J" where J is $(CR^7R^{7'})$n and n=0–3, J' is a bond or O, S, S═O, SO_2, NH, NR^7, $CR^7R^{7'}$, $R^2P═O$, $R^2P═S$, $R^2OP═O$, $R^2NHP═O$, $OP═OOR^2$, $OP═ONHR^2$, OSO_2, NHNH, $NHNR^6$, NR^6NH, N═N, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, or heterocyclo or substituted heterocyclo, and J" is $(CR^7R^{7'})$n and n=0–3, where Y is not a bond; and

W is $CR^7R^{7'}—CR^7R^{7'}$, $CR^7R^{7'}—C═O$, $NR^9—CR^7R^{7'}$, $N═CR^8$, N═N, $NR^9—NR^{9'}$, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, or aryl or substituted aryl, wherein,

when W' is not $NR^9—CR^7R^{7'}$, $N═CR^8$, N═N, $NR^9—NR^{9'}$, or heterocyclo or substituted heterocyclo, then J' must be O, S, S═O, SO_2, NH, NR^7, $OP═OOR^2$, $OP═ONHR^2$, OSO_2, NHNH, $NHNR^6$, NR^6NH, or N═N; or alternatively,

Y' is $CR^7R^{7'}—C═O$ and W' is $NR^9—CR^7R^{7'}$;

L is a bond; and

A_1 and A_2 are as defined above, especially where A_1 and/or A_2 are alkyl or optionally substituted alkyl (preferred such optional substituents being one or more groups V^1 defined below), with the proviso that, when Y'=O and W'=$—CH_2—CH_2—$, then at least one of A_1 or A_2 is not CH;

with the further provisos (2), (3), (6), (7) and (8) above.

The compounds of formula I and salts thereof comprise a core which can serve as a steroid mimic (and do not require the presence of a steroid-type (e.g., cyclopentanoperhydrophenanthrene analog) structure).

FURTHER DESCRIPTION OF THE INVENTION

The following are definitions of terms used in the present specification. The initial definition provided for a group or term herein applies to that group or term throughout the present specification individually or as part of another group, unless otherwise indicated.

The terms "alkyl" and "alk" refers to a straight or branched chain alkane (hydrocarbon) radical containing from 1 to 12 carbon atoms, preferably 1 to 6 carbon atoms. Exemplary such groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, isobutyl, pentyl, hexyl, isohexyl, heptyl, 4,4-dimethylpentyl, octyl, 2,2,4-trimethylpentyl, nonyl, decyl, undecyl, dodecyl, and the like. "Substituted alkyl" refers to an alkyl group substituted with one or more substituents, preferably 1 to 4 substituents, at any available point of attachment. Exemplary substituents include but are not limited to one or more of the following groups: halo (e.g., a single halo substituent or multiple halo substitutents forming, in the latter case, groups such as a perfluoroalkyl group or an alkyl group bearing Cl_3 or CF_3), alkoxy, alkylthio, hydroxy, carboxy (i.e., —COOH), alkoxycarbonyl, alkylcarbonyloxy, amino (i.e., $—NH_2$), carbamoyl or substituted carbomoyl, carbamate or substituted carbamate, urea or substituted urea, amidinyl or substituted amidinyl, thiol (i.e., —SH), aryl,

heterocycle, cycloalkyl, heterocycloalkyl, —S-aryl, —S-heterocycle, —S═O-aryl, —S═O-heterocycle, arylalkyl-O—, —$S(O)_2$-aryl, —$S(O)_2$-heterocycle, —NHS$(O)_2$-aryl, —NHS$(O)_2$-heterocycle, —NHS$(O)_2$NH-aryl, —NHS$(O)_2$NH-heterocycle, —$P(O)_2$-aryl, —$P(O)_2$-heterocycle, —NHP$(O)_2$-aryl, —NHP$(O)_2$-heterocycle, —NHP$(O)_2$NH-aryl, —NHP$(O)_2$NH-heterocycle, —O-aryl, —O-heterocycle, —NH-aryl, —NH-heterocycle, —NHC═O-aryl, —NHC═O-alkyl, —NHC═O-heterocycle, —OC═O-aryl, —OC═O-heterocycle, —NHC═ONH-aryl, —NHC═ONH-heterocycle, —OC═OO-aryl, —OC═OO-heterocycle, —OC═ONH-aryl, —OC═ONH-heterocycle, —NHC↑OO-aryl, —NHC═OO-heterocycle, —NHC═OO-alkyl, —C═ONH-aryl, —C═ONH-heterocycle, —C═OO-aryl, —C═OO-heterocycle, —N(alkyl)$S(O)_2$-aryl, —N(alkyl)S$(O)_2$-heterocycle, —N(alkyl)$S(O)_2$NH-aryl, —N(alkyl)S$(O)_2$ NH-heterocycle, —N(alkyl)$P(O)_2$-aryl, —N(alkyl)P$(O)_2$-heterocycle, —N(alkyl)$P(O)_2$NH-aryl, —N(alkyl)P$(O)_2$ NH-heterocycle, —N(alkyl)-aryl, —N(alkyl)-heterocycle, —N(alkyl)C═O-aryl, —N(alkyl)C═O-heterocycle, —N(alkyl)C═ONH-aryl, —N(alkyl)C═ONH-heterocycle, —OC═ON(alkyl)-aryl, —OC═ON(alkyl)-heterocycle, —N(alkyl)C═OO-aryl, —N(alkyl)C═OO-heterocycle, —C═ON(alkyl)-aryl, —C═ON(alkyl)-heterocycle, —NHS$(O)_2$N(alkyl)-aryl, —NHS(O)$_2$N(alkyl)-heterocycle, —NHP$(O)_2$N(alkyl)-aryl, NHP(O)$_2$N(alkyl)-heterocycle, —NHC═ON(alkyl)-aryl, —NHC═ON(alkyl)-heterocycle, —N(alkyl)$S(O)_2$N(alkyl)-aryl, —N(alkyl)$S(O)_2$N(alkyl)-heterocycle, —N(alkyl)$P(O)_2$N(alkyl)-aryl, —N(alkyl)$P(O)_2$ N(alkyl)-heterocycle, —N(alkyl)C═ON(alkyl)-aryl, and —N(alkyl)C═ON(alkyl)-heterocycle. In the aforementioned exemplary substitutents, in each instance, groups such as "alkyl", "aryl" and "heterocycle" can themselves be optionally substituted; for example, "alkyl" in the group "NCH═OO-alkyl" recited above can be optionally substituted so that both "NHC═OO-alkyl" and "NHC═OO-substituted alkyl" are exemplary substitutents. Exemplary alkyl substituents also include groups such as "T" and "T—R^{12}" (which are defined below), especially for substituted alkyl groups within A_1 or A_2.

The term "alkenyl" refers to a straight or branched chain hydrocarbon radical containing from 2 to 12 carbon atoms and at least one carbon—carbon double bond. Exemplary such groups include ethenyl or allyl. "Substituted alkenyl" refers to an alkenyl group substituted with one or more substituents, preferably 1 to 4 substituents, at any available point of attachment. Exemplary substituents include, but are not limited to, alkyl or substituted alkyl, as well as those groups recited above as exemplary alkyl substituents.

The term "alkynyl" refers to a straight or branched chain hydrocarbon radical containing from 2 to 12 carbon atoms and at least one carbon to carbon triple bond. Exemplary such groups include ethynyl. "Substituted alkynyl" refers to an alkynyl group substituted with one or more substituents, preferably 1 to 4 substituents, at any available point of attachment. Exemplary substituents include, but are not limited to, alkyl or substituted alkyl, as well as those groups recited above as exemplary alkyl substituents.

The term "cycloalkyl" refers to a fully saturated cyclic hydrocarbon group containing from 1 to 4 rings and 3 to 8 carbons per ring. Exemplary such groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, etc. "Substituted cycloalkyl" refers to a cycloalkyl group substituted with one or more substituents, preferably 1 to 4 substituents, at any available point of attachment. Exemplary substituents include, but are not limited to, nitro, cyano, alkyl or substituted alkyl, as well as those groups recited above as exemplary alkyl substituents, and as previously mentioned as preferred aryl substituents in the definition for G. Exemplary substituents also include spiro-attached or fused cyclic substituents, especially cycloalkenyl or substituted cycloalkenyl.

The term "cycloalkenyl" refers to a partially unsaturated cyclic hydrocarbon group containing 1 to 4 rings and 3 to 8 carbons per ring. Exemplary such groups include cyclobutenyl, cyclopentenyl, cyclohexenyl, etc. "Substituted cycloalkenyl" refers to a cycloalkenyl group substituted with one more substituents, preferably 1 to 4 substituents, at any available point of attachment. Exemplary substituents include but are not limited to nitro, cyano, alkyl or substituted alkyl, as well as those groups recited above as exemplary alkyl substituents, and as previously mentioned as preferred aryl substituents in the definition for G. Exemplary substituents also include spiro-attached or fused cyclic substituents, especially cycloalkyl or substituted cycloalkyl.

The terms "alkoxy" or "alkylthio" refer to an alkyl group as described above bonded through an oxygen linkage (—O—) or a sulfur linkage (—S—), respectively. The terms "substituted alkoxy" or "substituted alkylthio" refer to a substituted alkyl group as described above bonded through an oxygen or sulfur linkage, respectively.

The term "alkoxycarbonyl" refers to an alkoxy group bonded through a carbonyl group.

The term "alkylcarbonyl" refers to an alkyl group bonded through a carbonyl group. The term "alkylcarbonyloxy" refers to an alkylcarbonyl group bonded through an oxygen linkage.

The terms "arylalkyl", "substituted arylalkyl," "cycloalkylalkyl," "substituted cycloalkylalkyl," "cycloalkenylalkyl", "substituted cycloalkenylalkyl", "heterocycloalkyl" and "substituted heterocycloalkyl" refer to aryl, cycloalkyl, cycloalkenyl and heterocyclo groups bonded through an alkyl group, substituted on the aryl, cycloalkyl, cycloalkenyl or heterocyclo and/or the alkyl group where indicated as "substituted."

The term "aryl" refers to cyclic, aromatic hydrocarbon groups which have 1 to 5 aromatic rings, especially monocyclic or bicyclic groups such as phenyl, biphenyl or naphthyl. Where containing two or more aromatic rings (bicyclic, etc.), the aromatic rings of the aryl group may be joined at a single point (e.g., biphenyl), or fused (e.g., naphthyl, phenanthrenyl and the like). "Substituted aryl" refers to an aryl group substituted by one or more substituents, preferably 1, 2, 3, 4 or 5 substituents, at any point of attachment. Exemplary substituents include, but are not limited to, nitro, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, cyano, alkyl-$S(O)_m$— (m=0, 1 or 2), alkyl or substituted alkyl, as well as those groups recited above as exemplary alkyl substituents and as previously mentioned as preferred aryl substituents in the definition for G. Exemplary substituents also include fused cyclic substituents, such as heterocyclo or cycloalkenyl, or substituted heterocyclo or cycloalkenyl, groups (e.g., thereby forming a fluoroenyl, tetrahydronapthalenyl, or dihydroindenyl group).

"Carbamoyl" refers to the group —CONH— which is bonded on one end to the remainder of the molecule and on the other to hydrogen or an organic moiety (such as alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, alkylcarbonyl, hydroxyl and substituted nitrogen). "Carbamate" refers to the group —O—CO—NH— which is bonded

on one end to the remainder of the molecule and on the other to hydrogen or an organic moiety (such as those listed above). "Urea" refers to the group —NH—CO—NH— which is bonded on one end to the remainder of the molecule and on the other to hydrogen or an organic moiety (such as those listed above). "Amidinyl" refers to the group —C(═NH)(NH_2). "Substituted carbamoyl," "substituted carbamate," "substituted urea" and "substituted amidinyl" refer to carbamoyl, carbamate, urea or amidinyl groups as described above in which one more of the hydrogen groups are replaced by an organic moiety (such as those listed above).

The terms "heterocycle", heterocyclic" and "heterocyclo" refer to fully saturated, or partially or fully unsaturated, including aromatic (i.e., "heteroaryl") cyclic groups (for example, 3 to 7 membered monocyclic, 7 to 11 membered bicyclic, or 10 to 16 membered tricyclic ring systems) which have at least one heteroatom in at least one carbon atom-containing ring. Each ring of the heterocyclic group containing a heteroatom may have 1, 2, 3, or 4 heteroatoms selected from nitrogen atoms, oxygen atoms and/or sulfur atoms, where the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatoms may optionally be quaternized. (The term "heteroarylium" refers to a heteroaryl group bearing a quaternary nitrogen atom and thus a positive charge.) The heterocyclic group may be attached to the remainder of the molecule at any heteroatom or carbon atom of the ring or ring system. Exemplary monocyclic heterocyclic groups include ethylene oxide, azetidinyl, pyrrolidinyl, pyrrolyl, pyrazolyl, oxetanyl, pyrazolinyl, imidazolyl, imidazolinyl, imidazolidinyl, oxazolyl, oxazolidinyl, isoxazolinyl, isoxazolyl, thiazolyl, thiadiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, furyl, tetrahydrofuryl, thienyl, oxadiazolyl, piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, hexahydrodiazepinyl, 4-piperidonyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, triazolyl, tetrazolyl, tetrahydropyranyl, morpholinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, 1,3-dioxolane and tetrahydro-1,1-dioxothienyl, and the like. Exemplary bicyclic heterocyclic groups include indolyl, isoindolyl, benzothiazolyl, benzodioxolyl, benzoxazolyl, benzoxadiazolyl, benzothienyl, quinuclidinyl, quinolinyl, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuryl, benzofurazanyl, chromonyl, coumarinyl, benzopyranyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridinyl (such as furo[2,3-c]pyridinyl, furo[3,2-b]pyridinyl] or furo[2,3-b] pyridinyl), dihydrobenzodioxinyl, dihydrodioxidobenzothiophenyl, dihydroisoindolyl, dihydroindolyl, dihydroquinolinyl, dihydroquinazolinyl (such as 3,4-dihydro-4-oxo-quinazolinyl), triazinylazepinyl, tetrahydroquinolinyl and the like. Exemplary tricyclic heterocyclic groups include carbazolyl, benzidolyl, phenanthrolinyl, dibenzofuranyl, acridinyl, phenanthridinyl, xanthenyl and the like.

"Substituted heterocycle," "substituted heterocyclic," and "substituted heterocyclo" (such as "substituted heteroaryl") refer to heterocycle, heterocyclic or heterocyclo groups substituted with one or more substituents, preferably 1 to 4 substituents, at any available point of attachment. Exemplary substituents include, but are not limited to, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, nitro, oxo (i.e., —O), cyano, alkyl-S(O)$_m$— (m=0, 1 or 2), alkyl or substituted alkyl, as well as those groups recited above as exemplary alkyl substituents, and as previously mentioned as preferred heterocyclo substituents in the definition for G.

The term "quaternary nitrogen" refers to a tetravalent positively charged nitrogen atom including, for example, the positively charged nitrogen in a tetraalkylammonium group (e.g., tetramethylammonium, N-methylpyridinium), the positively charged nitrogen in protonated ammonium species (e.g., trimethylhydroammonium, N-hydropyridinium), the positively charged nitrogen in amine N-oxides (e.g., N-methyl-morpholine-N-oxide, pyridine-N-oxide), and the positively charged nitrogen in an N-amino-ammonium group (e.g., N-aminopyridinium).

The terms "halogen" or "halo" refer to chlorine, bromine, fluorine or iodine.

The terms "hydroxylamine" and "hydroxylamide" refer to the groups OH—NH— and OH—NH—CO—, respectively.

When a functional group is termed "protected", this means that the group is in modified form to mitigate, especially preclude, undesired side reactions at the protected site. Suitable protecting groups for the methods and compounds described herein include, without limitation, those described in standard textbooks, such as Greene, T. W. et al., *Protective Groups in Organic Synthesis*, Wiley, N.Y. (1991).

When a term such as "(CRR)n" is used, it denotes an optionally substituted alkyl chain existing between the two fragments to which it is bonded, the length of which chain is defined by the range described for the term n. An example of this is n=0–3, implying from zero to three (CRR) units existing between the two fragments, which are attached to the primary and terminal (CRR) units. In the situation where the term n is set to zero (n=0) then a bond exists between the two fragments attached to (CRR).

Unless otherwise indicated, any heteroatom with unsatisfied valences is assumed to have hydrogen atoms sufficient to satisfy the valences.

Divalent groups, such as those in the definition of W (e.g., $NR^9—CR^7R^{7'}$), may be bonded in either direction to the remainder of the molecule (e.g,

$$—\underset{|}{A_1}—NR^9—CR^7R^{7'}—\underset{|}{A_2}— \quad \text{or,} \quad —\underset{|}{A_1}—CR^7R^{7'}—NR^9—\underset{|}{A_2}—$$

for the aforementioned group within the definition of W).

Carboxylate anion refers to a negatively charged group —COO^-.

The compounds of formula I form salts which are also within the scope of this invention. Reference to a compound of the formula I herein is understood to include reference to salts thereof, unless otherwise indicated. The term "salt(s)", as employed herein, denotes acidic and/or basic salts formed with inorganic and/or organic acids and bases. In addition, when a compound of formula I contains both a basic moiety, such as but not limited to a pyridine or imidazole, and an acidic moiety such as but not limited to a carboxylic acid, zwitterions ("inner salts") may be formed and are included within the term "salt(s)" as used herein. Pharmaceutically acceptable (i.e., non-toxic, physiologically acceptable) salts are preferred, although other salts are also useful, e.g., in isolation or purification steps which may be employed during preparation. Salts of the compounds of the formula I may be formed, for example, by reacting a compound I with an amount of acid or base, such as an equivalent amount, in a medium such as one in which the salt precipitates or in an aqueous medium followed by lyophilization.

The compounds of formula I which contain a basic moiety, such as but not limited to an amine or a pyridine or

imidazole ring, may form salts with a variety of organic and inorganic acids. Exemplary acid addition salts include acetates (such as those formed with acetic acid or trihaloacetic acid, for example, trifluoroacetic acid), adipates, alginates, ascorbates, aspartates, benzoates, benzenesulfonates, bisulfates, borates, butyrates, citrates, camphorates, camphorsulfonates, cyclopentanepropionates, digluconates, dodecylsulfates, ethanesulfonates, fumarates, glucoheptanoates, glycerophosphates, hemisulfates, heptanoates, hexanoates, hydrochlorides, hydrobromides, hydroiodides, hydroxyethanesulfonates (e.g., 2-hydroxyethanesulfonates), lactates, maleates, methanesulfonates, naphthalenesulfonates (e.g., 2-naphthalenesulfonates), nicotinates, nitrates, oxalates, pectinates, persulfates, phenylpropionates (e.g., 3-phenylpropionates), phosphates, picrates, pivalates, propionates, salicylates, succinates, sulfates (such as those formed with sulfuric acid), sulfonates (such as those mentioned herein), tartrates, thiocyanates, toluenesulfonates such as tosylates, undecanoates, and the like.

The compounds of formula I which contain an acidic moiety, such but not limited to a carboxylic acid, may form salts with a variety of organic and inorganic bases. Exemplary basic salts include ammonium salts, alkali metal salts such as sodium, lithium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases (for example, organic amines) such as benzathines, dicyclohexylamines, hydrabamines (formed with N,N-bis(dehydroabietyl)ethylenediamine), N-methyl-D-glucamines, N-methyl-D-glycamides, t-butyl amines, and salts with amino acids such as arginine, lysine and the like. Basic nitrogen-containing groups may be quaternized with agents such as lower alkyl halides (e.g. methyl, ethyl, propyl, and butyl chlorides, bromides and iodides), dialkyl sulfates (e.g. dimethyl, diethyl, dibutyl, and diamyl sulfates), long chain halides (e.g. decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides), aralkyl halides (e.g. benzyl and phenethyl bromides), and others.

Prodrugs and solvates of the compounds of the invention are also contemplated herein. The term "prodrug" as employed herein denotes a compound which, upon administration to a subject, undergoes chemical conversion by metabolic or chemical processes to yield a compound of the formula I, or a salt and/or solvate thereof. Solvates of the compounds of formula I include, for example, hydrates.

Compounds of the formula I, and salts thereof, may exist in their tautomeric form (for example, as an amide or imino ether). All such tautomeric forms are contemplated herein as part of the present invention.

All stereoisomers of the present compounds (for example, those which may exist due to asymmetric carbons on various substituents), including enantiomeric forms and diastereomeric forms, are contemplated within the scope of this invention. Individual stereoisomers of the compounds of the invention may, for example, be substantially free of other isomers (e.g., as a pure or substantially pure optical isomer having a specified activity), or may be admixed, for example, as racemates or with all other, or other selected, stereoisomers. The chiral centers of the present invention may have the S or R configuration as defined by the IUPAC 1974 Recommendations. The racemic forms can be resolved by physical methods, such as, for example, fractional crystallization, separation or crystallization of diastereomeric derivatives or separation by chiral column chromatography. The individual optical isomers can be obtained from the racemates by any suitable method, including without limitation, conventional methods, such as, for example, salt formation with an optically active acid followed by crystallization.

All configurational isomers of the compounds of the present invention are contemplated, either in admixture or in pure or substantially pure form. The definition of compounds of the present invention embraces both cis (Z) and trans (E) alkene isomers, as well as cis and trans isomers of cyclic hydrocarbon or heterocyclo rings. In certain cases, for example, the exo or endo conformation can be preferred for the fused ring system bonded to G—L in formula I. For example, for androgen receptor antagonists (or selective androgen receptor modulators), where Y is O or NR^7, the exo configuration can be preferred, while for most other definitions of Y, the endo configuration can be preferred. As can be appreciated, the preferred configuration can be a function of the particular compound and its preferred activity. Separation of configurational isomers can be achieved by any suitable method, such as column chromatography.

Throughout the specifications, groups and substituents thereof may be chosen to provide stable moieties and compounds.

Embodiments indicated herein as exemplary or preferred are intended to be illustrative and not limiting.

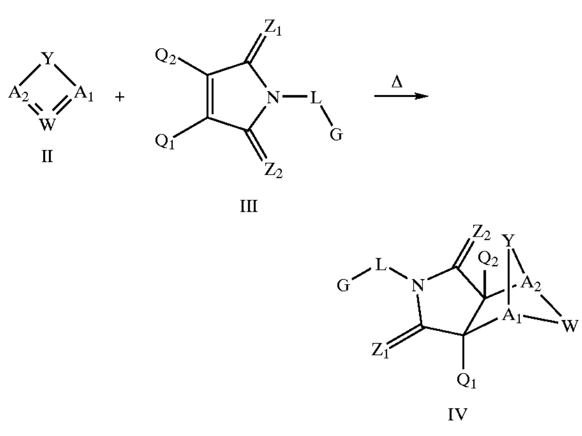
Methods of Preparation

The compounds of the present invention may be prepared by methods such as those illustrated in the following Schemes I to XI. Solvents, temperatures, pressures, and other reaction conditions may readily be selected by one of ordinary skill in the art. Starting materials are commercially available or readily prepared by one of ordinary skill in the art. Combinatorial techniques may be employed in the preparation of compounds, for example, where the intermediates possess groups suitable for these techniques. See the following which describe other methods which may be employed in the preparation of compounds of the present invention: Li, et al., *Eur. J. Org. Chem.* 9, 1841–1850 (1998); Li, Y-Q, *Synlett.* 5, 461–464 (1996); Thiemann, et al., *Bull. Chem. Soc. Jpn.* 67, 1886–1893 (1994); Tsuge et al., *Heterocycles* 14, 423–428 (1980); Ward et al., *Can J. Chem.* 75, 681–693 (1997); Ward et al., *Can J. Chem.* 69, 1487–1497 (1991); Ward et al., *Tetrahedron Lett.* 31, 845–848 (1990); Fleming et al., *J. Org. Chem.* 44, 2280–2282 (1979); Jankowski et al., *J. Organomet. Chem.* 595, 109–113 (2000); Keglevich et al., *J. Organomet. Chem.* 579, 182–189 (1999); Keglevich et al., *J. Organomet. Chem.* 570, 49–539 (1998); Jankowski et al., *Hetroat. Chem.* 7, 369–374 (1996); Jankowski et al., *J. Am. Chem. Soc.* 113, 7011–7017 (1991); Quin et al., *Tetrahedron Lett.* 31, 6473–6476 (1990); Quin et al., *J. Org. Chem.* 59, 120–129 (1994); Quin et al., *J. Org. Chem.* 58, 6212–6216 (1993); Quin et al., *Phosphorous, Sulfur Silicon Relat. Elem.* 63, 349–362 (1991); Quin et al., *Hetroat. Chem.* 2, 359–367 (1991); Hussong et al., *Phosphorus Sulfur.* 25, 201–212 (1985); Quin et al., *J. Org. Chem.* 51, 3341–3347 (1986); Myers et al., *J. Am. Chem. Soc.* 114, 5684–5692 (1992); Myers et al., *J. Am. Chem. Soc.* 113, 6682–6683 (1991); Shen et al., U.S. Pat. No. 5,817,679; Cordone et al., *J. Am. Chem. Soc.* 111, 5969–5970 (1989); Jung et al., *J. Chem. Soc. Commun.* 630–632 (1984); Lay et al., *J. Am. Chem. Soc.* 104, 7658–7659 (1982); Gonzalez et al., *J. Am. Chem. Soc.* 117, 3405–3421 (1995); Kreher et al., *Chem Ber.* 125, 183–189 (1992); Simig et al., *Synlett.* 7, 425–426 (1990); Sha et al., *J. Org. Chem.* 55, 2446–2450 (1990); Drew et al., *J. Chem. Soc., Perkin Trans.* 1 7, 1277–1284 (1985); Kreher et al., *Anorg. Chem., Org Chem.* 31B, 599–604 (1976); Avalos et al., *Tetrahedron Lett.* 39, 9301–9304 (1998); Gousse et al., *Macromolecules* 31, 314–321 (1998); Mikhailyuchenko et al., *Khim. Geterotsikl. Soedin.* 6, 751–758

(1993); Lubowitz et al., U.S. Pat. No. 4,476,184; Padwa et al., *J. Org. Chem.* 61, 3706–3714 (1996); Schlessinger et al., *J. Org. Chem.* 59, 3246–3247 (1994); Buchmeiser et al., WO Publication No. 9827423; Tanabe et al., Japanese Patent Document JP 07144477; Mochizucki et al., Japanese Patent Document JP 63170383; Hosoda et al., Japanese Patent Document JP 62053963; Onaka et al., Japanese Patent Document JP 62053964; Kato et al., Japanese Patent Document JP 53086035; Kato et al., Japanese Patent Document JP 51088631; Tottori et al., Japanese Patent Document JP 49124225; Augustin et al., German Patent Document DD101271; Title et al., French Patent Document FR 2031538; Gousse et al., *Polym. Int.* 48, 723–731 (1999); Padwa et al., *J. Org. Chem.* 62, 4088–4096 (1997); Theurillat-Moritz et al., *Tetrahedron: Asymmetry* 7, 3163–3168 (1996); Mathews et al., *J. Carbohydr. Chem.* 14, 287–97 (1995); Srivastava et al., *Natl. Acad. Sci. Lett.* (*India*) 15, 41–44 (1992); Mayorga et al., *Rev. Cubana Quim.* 4, 1–6 (1988); Kondoli et al., *J. Chem. Res., Synop.* 3, 76 (1987); Primelles et al., *Cent. Azucar* 7–14 (1985); Solov'eva et al., *Khim. Geterotsikl. Soedin.* 5, 613–15 (1984); Liu et al., *Yaoxue Xuebao* 18, 752–759 (1983); Joshi et al., *Indian J. Chem, Sect. B.* 22B, 131–135 (1983); Amos et al., WO Publication No. 9829495; Odagiri et al., U.S. Pat. No. 4,670,536; Gallucci et al., European Patent Document EP 355435; Redmore, D. U.S. Pat. No. 3,821,232; Nakano et al., *Heterocycles* 35, 37–40 (1993); Tomisawa et al., *Chem. Pharm. Bull.* 36, 1692–1697 (1988); Krow et al., *J. Heterocycl. Chem.* 22, 131–135 (1985); Krow et al., *J. Org. Chem.* 47, 1989–1993 (1982); Liu et al., *Yaoxue Xuebao* 18, 752–759 (1983); Nishikawa et al., *Yaoxue Xuebao* JP 01061457; and/or Rice et al., *J. Med. Chem.* 11, 183–185 (1968).

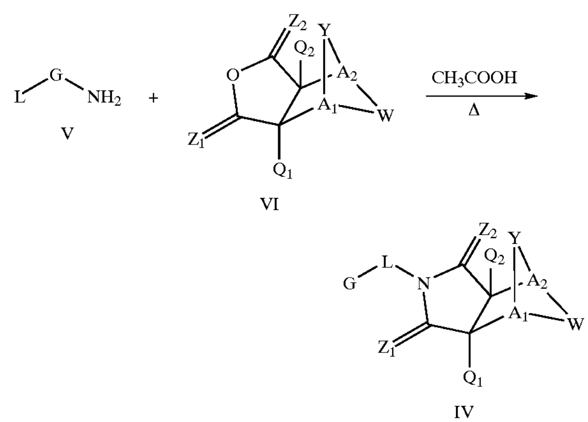
All documents cited in the present specification, such as those cited in this "Methods of Preparation" as well as other sections herein, are incorporated herein by reference in their entirety. Reference to any document herein is not to be construed as an admission that such document is prior art.

Scheme I



As illustrated in Scheme I, a diene of formula II can be reacted with a dienophile of formula III, under conditions readily selected by one skilled in the art (such as by the addition of heat ("Δ")), to obtain a compound of formula IV, which is a compound of formula I. An intermediate diene of formula II can be obtained from commercial sources or readily made by one skilled in the art, for example, in accordance with the following literature documents and the references found therein: Hofman et al., *J. Agric. Food Chem.* 45, 898–906 (1997); Baciocchi et al., *J. Chem. Soc., Perkin Trans.* 2 8, 821–824 (1975); Wu et al., *J. Heterocycles* 38, 1507–1518 (1994); Yin et al., *Tetrahedron Lett.* 38, 5953–5954 (1997); Mic'ovic' et al., *Tetrahedron* 20, 2279–2287 (1964); Gorbunova et al., *J. Org. Chem.* 35, 1557–1566 (1999); Rassu et al., *Chem. Soc. Rev.* 29, 109–118 (2000); Kaberdin et al., *Russ. Chem. Rev.* 68, 765–779 (1999); Barluenga et al., *Aldrichimica Acta* 32, 4–15 (1999); Bogdanowicz-Szwed et al., *Pol. Wiad. Chem.* 52, 821–842 (1998); Casiraghi et al., *Adv. Asymmetric Synth.* 3, 113–189 (1998); and/or Baeckvall et al., *Chem. Rev.* 98, 2291–2312 (1998). An intermediate dieneophile of formula III can be obtained from commercial sources or readily made by one skilled in the art, for example, in accordance with the following literature references and the references found therein: Deshpande et al., *Heterocycles* 51, 2159–2162 (1999); Seijas et al., *J. Chem. Res., Synop.* 7, 420421 (1999); Langer et al., *Eur. J. Org. Chem.* 7, 1467–1470 (1998); Kita et al., Japanese Patent Document JP 09194458; Lopez-Alvarado et al., *J. Org. Chem.* 61, 5865–5870 (1996); Condon et al., U.S. Pat. No. 5,523,277; Sasakihara et al., Japanese Patent Document JP 04290868; Igarashi et al., Japanese Patent Document JP 04149173; Aoyama et al., Japanese Patent Document JP 04134063; Aoyama et al., Japanese Patent Document JP 04134062; Pastor et al., *J. Org. Chem.* 53, 5776–5779 (1988); and/or Takahashi et al., *Chem. Lett.* 6, 1229–1232 (1987).

Scheme II

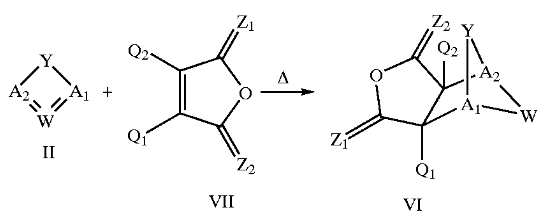


As illustrated in Scheme II, compounds of formula I can be obtained by reaction of a primary amine of formula IV with a substituted anhydride-like intermediate of formula VI, for example, in a solvent such as acetic acid with or without heating, to yield a compound of formula IV, which is a compound of formula I. Primary amines of formula V can be obtained from commercial sources or readily synthesized by one skilled in the art. Anhydride-like agents of formula VI can be obtained from commercial sources or readily synthesized by one skilled in the art. The documents listed following describe exemplary approaches for the synthesis of intermediates of formula VI as well as synthetic approaches which can be applied to the synthesis of compounds of formula IV (all incorporated herein by reference in their entirety): Kohler, E. P.; Tishler, M.; Potter, H.; Thompson, H. T. *J. Am. Chem. Soc.* 1939, 1057–1061; Yur'ev, Y. K.; Zefirov, N. S. *J. Gen. Chem. U.S.S.R.* (*Engl. Transl.*) 1961, 31, 772–5; Norman G. Gaylord U.S. Pat. No. 3,995,099; Schueler, P. E.; Rhodes, Y. E. *J. Org. Chem.* 1974, 39, 2063–9; Ishitobi, H.; Tanida, H; Tsuji, T. *Bull. Chem. Soc. Japan* 1971, 44, 2993–3000; Stájer, G.; Virág, M.; Szabó, A. E.; Bernáth, G.; Sohár, P.; Sillanpää, R. *Acta.*

Chem. Scand. 1996, 50, 922–30; Hart, H.; Ghosh, T. *Tetrahedron Lett.* 1988,29,881–884; Kato, M.; Yamamoto, S.; Yoshihara, T.; Furuichi, K; Miwa, T. *Chem. Lett.* 1987, 1823–1826; Kottwitz, J.; Vorbrüggen, H. *Synthesis* 1995, 636–637; Creary, X. *J. Org. Chem.* 1975, 40, 3326–3331; Alder, K.; Ache, H.-J.; Flock, F. H. *Chem. Ber.* 1960, 93, 1888–1895; Toder, B. H.; Branca, S. J.; Dieter, R. K.; Smith, A. B. III *Synth. Commun.* 1975, 5, 435439; Sprague, P. W.; Heikes, J. E.; Gougoutas, J. Z.; Malley, M. F.; Harris, D. N.; and/or Greenberg, R. *J. Med. Chem.* 1985, 28, 1580–1590.

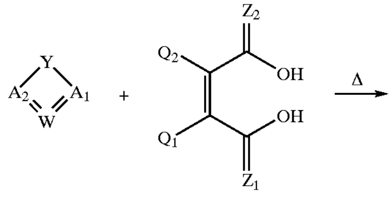
The aforementioned approach(es) can be applied in a combinatorial fashion, for example, by utilizing a multi-well reaction block such as is described in Waldemar Ruediger, Wen-Jeng Li, John W., Allen Jr., and Harold N. Weller III, U.S. Pat. No. 5,961,925, Apparatus for Synthesis of Multiple Organic Compounds With Pinch Valve Block (incorporated herein by reference in its entirety). By utilizing the above-mentioned multi-well reaction block, one can, for example, perform multiples of 96 reactions at a time. Solvent can then be removed from the reaction tubes without removal from the reaction block and the crude products can be precipitated using a base such as sodium bicarbonate. The precipitates can be collected by filtration of the reaction block and then the desired products can be transferred directly to 96 well plates for screening. In this fashion, a large array of compounds of formula I can be synthesized, and tests conducted as desired by an automated approach.

Scheme III



Scheme III describes a method for preparing an intermediate compound of formula VI which can be used to synthesize a compound of formula I, as described in Scheme II. As described in Scheme III, a diene of formula II can be reacted with a dieneophile of formula VII to yield the intermediate of formula VI. The methods applied to obtain such a transformation are analogous to those described in Scheme I.

Scheme IV



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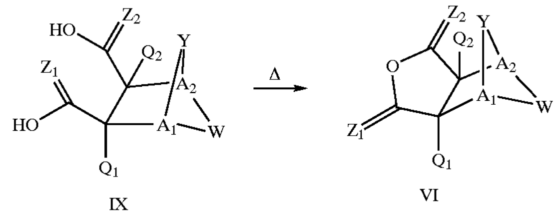
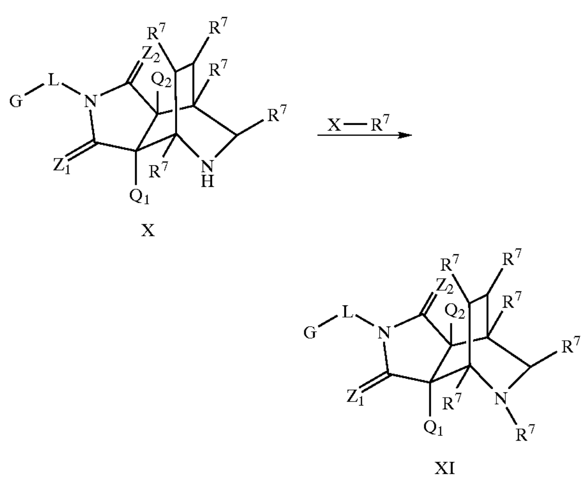


Scheme IV describes a method for preparing an intermediate compound of formula VI which can be used to synthesize a compound of formula I, as described in Scheme II. As shown in Scheme IV, a diene of formula II can be reacted with a dieneophile of formula VIII to yield the intermediate of formula IX. The intermediate of formula IX can be dehydrated to an anhydride-like intermediate of formula VI. Dehydration of the bis-acid intermediate of formula IX to can be achieved by a variety of methods, such as those known to one skilled in the art and described in the following documents and the references embodied therein: Sprague et al., *J. Med. Chem.* 28, 1580–1590 (1985); and/or Retemi et al., *J. Org. Chem.* 61, 6296–6301 (1996).

Schemes I to IV describe general methods for the synthesis of compounds of formula I, and intermediates thereof, in which substitution about the ring system is incorporated directly, for example, at the level of the intermediate diene, dienophile, anhydride-like intermediate and amine groups. In addition to these approaches, additional substitution can be incorporated onto an already-prepared compound of formula I by a variety of approaches to prepare other compounds of the formula I. Exemplary methods for further substitution are described in Schemes V to XI.

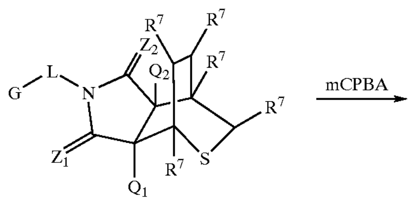
Scheme V



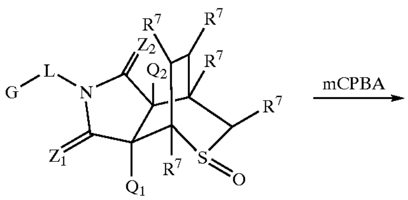
Scheme V describes one such approach to incorporating additional substitution into a structure of formula I. As illustrated in Scheme V, a compound of formula X, which is a compound of formula I where A_1 and A_2 are CR^7, W is $NH—CHR^7$ and Y is $CHR^7—CHR^7$, can be functionalized at the free amine of the group W by reaction with any of a variety of electrophilic agents such as acid halides or alkyl

halides in the presence of base, for example, by methods known by one skilled in the art. In Scheme V, X is a leaving group, and a compound of formula XI is a compound of formula I where A_1 and A_2 are CR^7, W is NR^7—CHR^7 and Y is CHR^7—CHR^7.

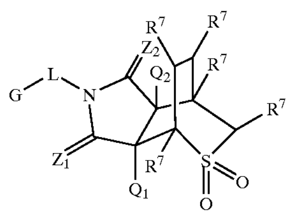
Scheme VI



XII



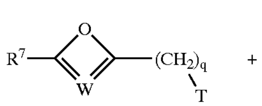
XIII



XIV

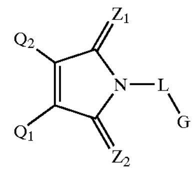
Scheme VI describes an additional approach for further incorporating substitution onto a compound of formula I. As illustrated in Scheme VI, a compound of formula XII, which is a compound of formula I where A_1 and A_2 are CR^7, W is S—CHR^7 and Y is CHR^7—CHR^7, can be partially oxidized with an oxidizing agent such as mCPBA or other agents such as those known to one skilled in the art, to give the sulfoxide analog of formula XIII, which is a compound of formula I where A_1 and A_2 are CR^7, W is SO—CHR^7 and Y is CHR^7—CHR^7. Further treatment of a compound of formula XIII with an oxidizing agent such as mCPBA or other agents such as those known to one skilled in the art, can yield the sulphone analog of formula XIV, which is a compound of formula I where A_1 and A_2 are CR^7, W is SO_2—CHR^7 and Y is CHR^7—CHR^7. Alternatively, a compound of formula XII can be converted directly to a compound of formula XIV by prolonged treatment with an oxidizing agent, such as mCPBA, or with other agents such as those known to one skilled in the art.

Scheme VII

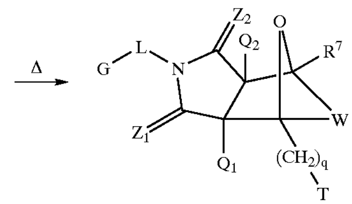


IIa

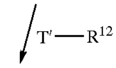
-continued

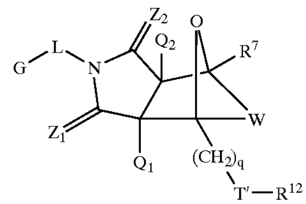


III

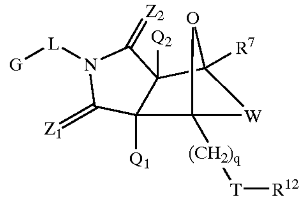


IVa





IVb

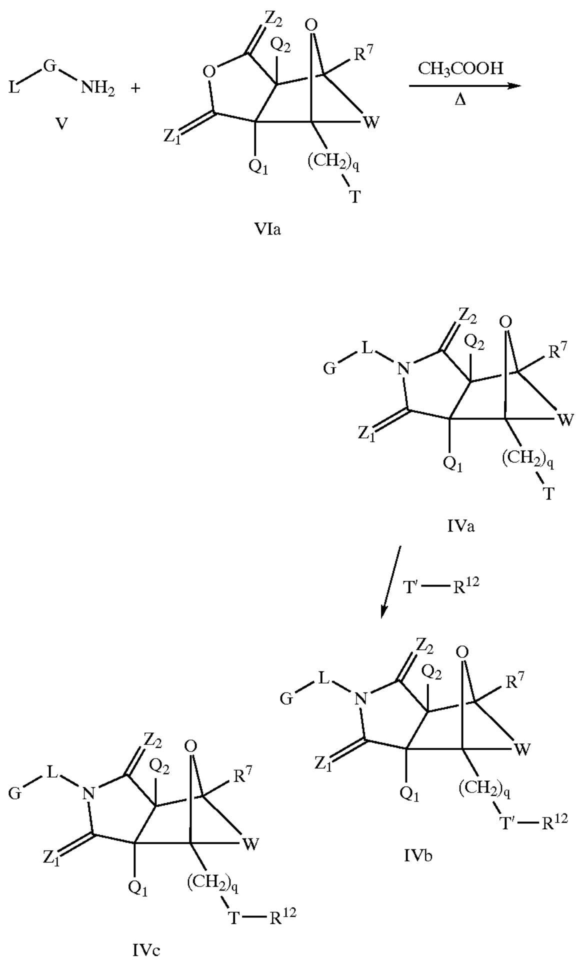


IVc

Scheme VII describes another approach to incorporating additional substitution onto a compound of formula I. As illustrated in Scheme VII, a diene of formula IIa can be reacted with a dienophile of formula III, as described in Scheme I, to yield a compound of formula IVa, which is a compound of formula I where Y is O, A_2 is CR^7 and A_1 is C—$(CH_2)_q$—T. The compound of formula IVa can be reacted with a reagent of formula R^{12}—T' to obtain a compound of formula IVb or IVc which are compounds of formula I where Y is O, A_2 is CR^7 and A_1 is C—$(CH_2)_q$—T'—R^{12} or C—$(CH_2)_q$—T—R^{12}, respectively. The reagent R^{12}—T' can be obtained from commercial sources or can readily be prepared by one skilled in the art.

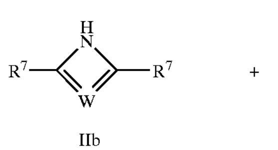
In the above Scheme, R^{12} has the same definition as R^7 defined earlier, q is zero or an integer from 0–8, and T is defined either as (1) a nucleophilic center such as, but not limited, to a nitrogen, oxygen or sulfur-containing group, capable of undergoing a nucleophilic substitution reaction with the leaving group T' or (2) a leaving group capable undergoing a nucleophilic substitution reaction with a nucleophilic group T' (such as, but not limited, to a nitrogen, oxygen or sulfur-containing nucleophilic group). T' has the same definition as T. In the present case, for example, a nucleophilic substitution reaction occurs when the attacking reagent (the nucleophile) brings an electron pair to the substrate, using this pair to form the new bond, and the leaving group (the nucleofuge) comes away with the electron pair, leaving as an anionic intermediate. For a detailed discussion of the mechanism of aliphatic nucleophilic substitutions and a review of specific aliphatic nucleophilic substitution reactions see *Advanced Organic Chemistry, Reactions, Mechanisms, and Structure,* 4^{th} *Addition.* Jerry March (Ed.), John Wiley & Sons, New York (1992) 293–500 and the references therein. Compounds of the formulae IVa, IVb, or IVc may, of course, be employed in the methods described herein (especially, in the treatment of nuclear hormone receptor-associated conditions) without undergoing further reaction of T or T'.

Scheme VIII



An alternate approach to compounds of formula IVa, IVb and IVc is illustrated in Scheme VIII. For this approach, techniques such as those described in Schemes II, III and IV can be applied to the preparation of an intermediate of formula VIa, where T and q are as defined in Scheme VII. The intermediate of formula VIa can be reacted with a substitited amine of formula V, as described in Scheme II, to yield the compound of formula IVa, which is a compound of formula I where Y is O, A_2 is CR^7 and A_1 is $C—CH_2)_q—T$. The compound of formula IVa can be treated in the manner described in Scheme VII to obtain compounds of formula IVb or IVc which are compounds of formula I where Y is O, A_2 is CR^7 and A_1 is $C—(CH_2)_q—T'—R^{12}$ or $C—(CH_2)_q—T—R^{12}$, respectively.

Scheme IX



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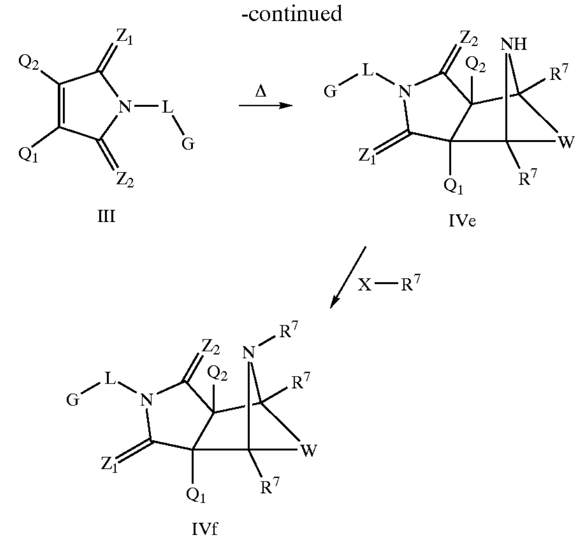
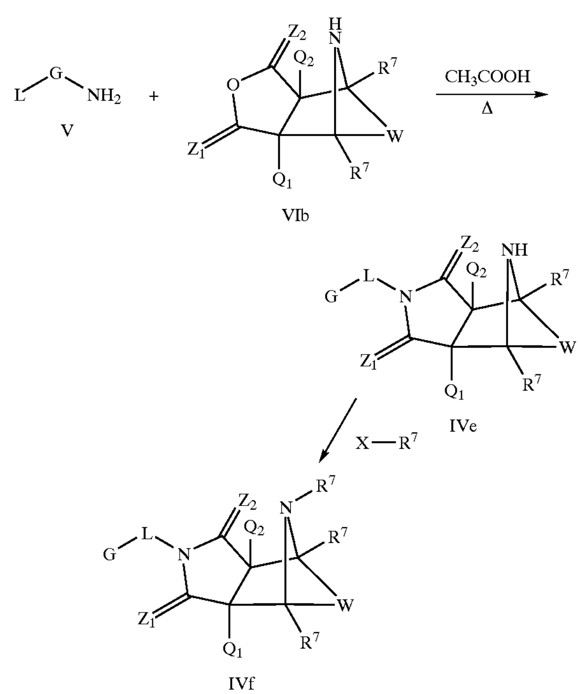


Scheme IX describes another approach to incorporating further substitution onto a compound of formula I. As illustrated in Scheme IX (where X is a leaving group), a diene of formula IIb can be reacted with a dienophile of formula III, as described in Scheme I, to yield a compound of formula IVe, which is a compound of formula I where Y is NH, and A_1 and A_2 are CR^7. The compound of formula IVe can be functionalized at the free amine by reacting with a variety of electrophilic agents such as acid halides or alkyl halides in the presence of base, for example by methods known by one skilled in the art and described in Scheme V, to yield a compound of formula IVf, which is a compound of formula I where Y is NR^7 and A_1 and A_2 are CR^7.

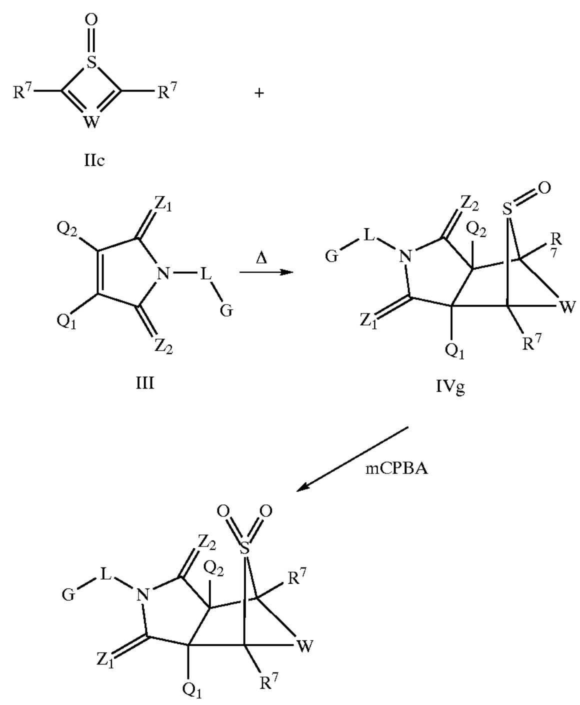
Scheme X



An alternate approach to compounds of formula IVe and IVf is illustrated in Scheme X. For this approach, techniques

as described in Schemes II, III and IV can be applied to the preparation of an intermediate of formula VIb. The intermediate of formula VIb can be reacted with a substituted amine of formula V, as described in Scheme II, to yield a compound of formula IVe, which is a compound of formula I where Y is NH, and A_1 and A_2 are CR^7. The latter intermediate can be treated in the manner described in Scheme V to obtain a compound of formula IVf, which is a compound of formula I where Y is NR^7, and A_1 and A_2 are CR^7.

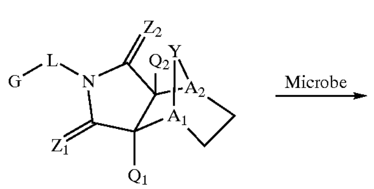
Scheme XI



IIc III IVg IVh

Scheme XI describes another approach to incorporating additional substitution onto a compound of formula I. As illustrated in Scheme XI, a diene of formula IIc can be reacted with a dienophile of formula III, as described in Scheme I, to yield a compound of formula IVg, which is a compound of formula I where Y is SO and A_1 and A_2 are CR^7. A compound of formula IVg can be treated with an oxidizing agent such as mCPBA, as described in Scheme VI, to yield a compound of formula IVh, which is a compound of formula I where Y is SO_2 and A_1 and A_2 are CR^7.

Scheme XII



XV

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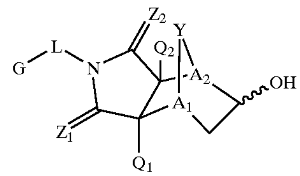
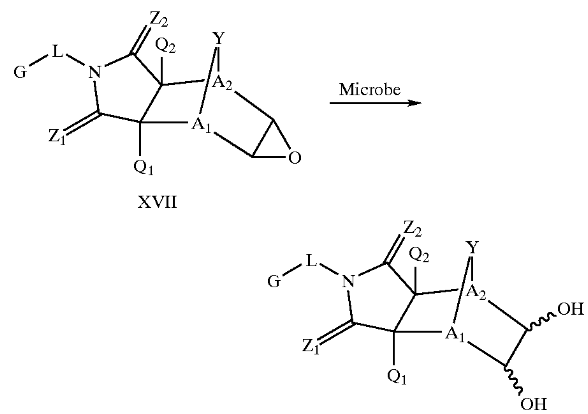


XVI

Scheme XII describes another approach to incorporating additional substitution onto a compound of formula I. As illustrated in Scheme XII, a compound of formula XV, which can be prepared in accordance with the above Schemes, can be incubated in the presence of a suitable enzyme or microorganism resulting in the formation of a hydroxylated analog of formula XVI. Such a process can be employed to yield regiospecific as well as enantiospecific incorporation of a hydroxyl group into a molecule of formula XV by a specific microorganism or by a series of different microorganisms. Such microorganisms can, for example, be bacterial, yeast or fungal in nature and can be obtained from distributors such as ATCC or identified for use in this method such as by methods known to one skilled in the art. Compound XVI is a compound of formula I where Y is as described above and A_1 and A_2 are preferably CR^7.

Scheme XIII



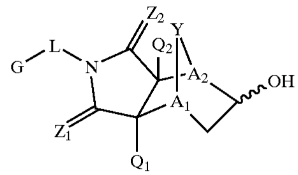
XVII XVIII

Scheme XIII describes another approach to incorporating additional substitution onto a compound of formula I. As illustrated in Scheme XIII, a compound of formula XVII, which can be prepared in accordance with the above Schemes, can be incubated in the presence of a suitable enzyme or microorganism resulting in the formation of a diol analog of formula XVIII. Such a process can be employed to yield regiospecific as well as enantiospecific transformation of a compound of formula XVII to a 1–2 diol of formula XVIII by a specific microorganism or by a series of different microorganisms. Such microorganisms can, for example, be bacterial, yeast or fungal in nature and can be obtained from distributors such as ATCC or identified for use in this method such as by methods known to one skilled in the art. Compound XVIII is a compound of formula I where Y is as described above and A_1 and A_2 are preferably CR^7.

The present invention also provides the methods of Schemes and XIII.

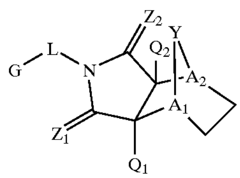
Thus, in one embodiment, the present invention provides a method for preparation of a compound of the following formula XVI, or salt thereof:

XVI



where the symbols are as defined herein,
comprising the steps of contacting a compound of the following formula XV, or salt thereof:

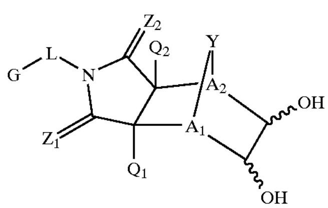
XV



where the symbols are as defined above;
with an enzyme or microorganism capable of catalyzing the hydroxylation of said compound XV to form said compound XVI, and effecting said hydroxylation.

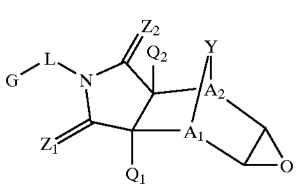
In another preferred embodiment, the present invention provides a method for preparation of a compound of the following formula XVIII, or salt thereof:

XVIII



where the symbols are as defined herein,
comprising the steps of contacting a compound of the following formula XVII, or salt thereof:

XVII



where the symbols are as defined above;
with an enzyme or microorganism capable of catalyzing the opening of the epoxide ring of compound XVII to form the diol of said compound XVIII, and effecting said ring opening and diol formation.

All stereoconfigurations of the unspecified chiral centers of the compounds of the formulae XV, XVI, XVII and XVIII are contemplated in the methods of the present invention, either alone (that is, substantially free of other stereoisomers) or in admixture with other stereoisomeric forms. Conversion of one isomer selectively (e.g., hydroxylation of the exo isomer preferentially to hydroxylation of the endo isomer) when contacting an isomeric mixture is a preferred embodiment of the invention. Conversion to one isomer selectively (e.g., hydroxylation on the exo face "exo isomer" preferentially to the endo face "endo isomer" or regioselective opening of an epoxide to form only one of two possible regioisomers of a trans diol) is a preferred embodiment of the invention. Hydroxylation of an achiral intermediate to form a single optical isomer of the hydroxylated product is also a preferred embodiment of the invention. Resolution of a recemic mixture of an intermediate by selective hydroxylation, or epoxide ring opening and diol formation, to generate one of the two possible optical isomers is also a preferred embodiment of the invention. The term "resolution" as used herein denotes partial, as well as, preferably, complete resolution.

The terms "enzymatic process" or "enzymatic method", as used herein, denote a process or method of the present invention employing an enzyme or microorganism. The term "hydroxylation", as used herein, denotes the addition of a hydroxyl group to a methylene group as described above. Hydroxylation can be achieved, for example, by contact with molecular oxygen according to the methods of the present invention. Diol formation can be achieved, for example, by contact with water according to the methods of the present invention. Use of "an enzyme or microorganism" in the present methods includes use of two or more, as well as a single, enzyme or microorganism.

The enzyme or microorganism employed in the present invention can be any enzyme or microorganism capable of catalyzing the enzymatic conversions described herein. The enyzmatic or microbial materials, regardless of origin or purity, can be employed in the free state or immobilized on a support such as by physical adsorption or entrapment. Microorganisms or enzymes suitable for use in the present invention can be selected by screening for the desired activity, for example, by contacting a candidate microorganism or enzyme with a starting compound XV or XVII or salt thereof, and noting conversion to the corresponding compound XVI or XVIII or salt thereof. The enzyme may, for example, be in the form of animal or plant enzymes or mixtures thereof, cells of microorganisms, crushed cells, extracts of cells, or of synthetic origin.

Exemplary microorganisms include those within the genera: *Streptomyces* or *Amycolatopsis*. Particularly preferred microorganisms are those within the species *Streptomyces griseus*, especially *Streptomyces griseus* ATCC 10137, and *Amycolatopsis orientalis* such as ATCC 14930, ATCC 21425, ATCC 35165, ATCC 39444, ATCC 43333, ATCC 43490, ATCC 53550, ATCC 53630, and especially ATCC 43491. The term "ATCC" as used herein refers to the accession number of the American Type Culture Collection, 10801 University Blvd., Manassas Va. 20110-2209, the depository for the organism referred to. It should be understood that mutants of these organisms are also contemplated by the present invention, for use in the methods described herein, such as those modified by the use of chemical, physical (for example, X-rays) or biological means (for example, by molecular biology techniques).

Preferred enzymes include those derived from microorganisms, particularly those microorganisms described above. Enzymes may be isolated, for example, by extraction and purification methods such as by methods known to those of ordinary skill in the art. An enzyme may, for example, be used in its free state or in immobilized form. One embodiment of the invention is that where an enzyme is adsorbed onto a suitable carrier, e.g., diatomaceous earth (porous Celite Hyflo Supercel), microporous polypropylene (Enka Accurel® polypropylene powder), or a nonionic poly-

meric adsorbent such as Amberlite® XAD-2 (polystyrene) or XAD-7 (polyacrylate) from Rohm and Haas Co. When employed to immobilize an enzyme, a carrier may control the enzyme particle size and prevent aggregation of the enzyme particles when used in an organic solvent. Immobilization can be accomplished, for example, by precipitating an aqueous solution of the enzyme with cold acetone in the presence of the Celite Hyflo Supercel followed by vacuum drying, or in the case of a nonionic polymeric adsorbent, incubating enzyme solutions with adsorbent on a shaker, removing excess solution and drying enzyme-adsorbent resins under vacuum. While it is desirable to use the least amount of enzyme possible, the amount of enzyme required will vary depending upon the specific activity of the enzyme used.

Hydroxylation as described above can occur in vivo. For example, liver enzyme can selectively, relative to the endo isomer, hydroxylate the exo isomer of a compound of the present invention. In conducting the methods of the present invention outside the body, liver microsomal hydroxylase can be employed as the enzyme for catalysis.

These processes may also be carried out using microbial cells containing an enzyme having the ability to catalyze the conversions. When using a microorganism to perform the conversion, these procedures are conveniently carried out by adding the cells and the starting material to the desired reaction medium.

Where microorganisms are employed, the cells may be used in the form of intact wet cells or dried cells such as lyophilized, spray-dried or heat-dried cells, or in the form of treated cell material such as ruptured cells or cell extracts. Cell extracts immobilized on Celite® or Accurel® polypropylene as described earlier may also be employed. The use of genetically engineered organisms is also contemplated. The host cell may be any cell, e.g. *Escherichia coli*, modified to contain a gene or genes for expressing one or more enzymes capable of catalysis as described herein.

Where one or more microorganisms are employed, the enzymatic methods of the present invention may be carried out subsequent to the fermentation of the microorganism (two-stage fermentation and conversion), or concurrently therewith, that is, in the latter case, by in situ fermentation and conversion (single-stage fermentation and conversion).

Growth of the microorganisms can be achieved by one of ordinary skill in the art by the use of an appropriate medium. Appropriate media for growing microorganisms include those which provide nutrients necessary for the growth of the microbial cells. A typical medium for growth includes necessary carbon sources, nitrogen sources, and elements (e.g. in trace amounts). Inducers may also be added. The term "inducer", as used herein, includes any compound enhancing formation of the desired enzymatic activity within the microbial cell.

Carbon sources can include sugars such as maltose, lactose, glucose, fructose, glycerol, sorbitol, sucrose, starch, mannitol, propylene glycol, and the like; organic acids such as sodium acetate, sodium citrate, and the like; and alcohols such as ethanol, propanol and the like:

Nitrogen sources can include N–Z amine A, corn steep liquor, soy bean meal, beef extracts, yeast extracts, molasses, baker's yeast, tryptone, nutrisoy, peptone; yeastamin, amino acids such as sodium glutamate and the like, sodium nitrate, ammonium sulfate and the like.

Trace elements can include magnesium, manganese, calcium, cobalt, nickel, iron, sodium and potassium salts. Phosphates may also be added in trace or, preferably, greater than trace amounts.

The medium employed can include more than one carbon or nitrogen source or other nutrient.

Preferred media for growth include aqueous media.

The agitation and aeration of the reaction mixture affects the amount of oxygen available during the conversion process when conducted, for example, in shake-flask cultures or fermentor tanks during growth of microorganisms.

Incubation of the reaction medium is preferably at a temperature between about 4 and about 60° C. The reaction time can be appropriately varied depending upon the amount of enzyme used and its specific activity. Reaction times may be reduced by increasing the reaction temperature and/or increasing the amount of enzyme added to the reaction solution.

It is also preferred to employ an aqueous liquid as the reaction medium, although an organic liquid, or a miscible or immiscible (biphasic) organic/aqueous liquid mixture, may also be employed. The amount of enzyme or microorganism employed relative to the starting material is selected to allow catalysis of the enzymatic conversions of the present invention.

Solvents for the organic phase of a biphasic solvent system may be any organic solvent immiscible in water, such as toluene, cyclohexane, xylene, trichlorotrifluoroethane and the like. The aqueous phase is conveniently of water, preferably deionized water, or a suitable aqueous buffer solution, especially a phosphate buffer solution. The biphasic solvent system preferably comprises between about 10 to 90 percent by volume of organic phase and between about 90 to 10 percent by volume of aqueous phase, and most preferably contains at or about 20 percent by volume of organic phase and at or about 80 percent by volume of the aqueous phase.

An exemplary embodiment of such processes starts with preparation of an aqueous solution of the enzyme(s) or microbes to be used. For example, the preferred enzyme(s) or microbes can be added to a suitable amount of an aqueous solvent, such as phosphate buffer or the like. This mixture is preferably adjusted to and maintained at a desired pH.

The compounds XVI and XVIII produced by the processes of the present invention can be isolated and purified, for example, by methods such as extraction, distillation, crystallization, and column chromatography.

Preferred Compounds

A preferred subgenus of the compounds of the present invention includes compounds of the formula I or salts thereof wherein one or more, preferably all, of the following substituents are as defined below:

G is an aryl or heterocyclo (e.g., heteroaryl) group, where said group is mono- or polycyclic, and which is optionally substituted at one or more positions, preferably with hydrogen, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, halo, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, aryl or substituted aryl, heterocyclo or substituted heterocyclo, arylalkyl or substituted arylalkyl, heterocycloalkyl or substituted heterocycloalkyl, CN, $R^1OC{=}O$, $R^1C{=}O$, $R^1HNC{=}O$, $R^1R^2NC{=}O$, $HOCR^3R^{3'}$, nitro, R^1OCH_2, R^1O, NH_2, NR^4R^5, $S{=}OR^1$, SO_2R^1, $SO_2NR^1R^{1'}$, $(R^1)(R^{1'})P{=}O$, or $(R^{1'})(NHR^1)P{=}O$;

Z_1 is O, S, NH, or NR^6;

Z_2 is O, S, NH, or NR^6;

A_1 is CR^7 or N;

A_2 is CR^7 or N;

Y is J—J'—J" where J is $(CR^7R^{7'})n$ and n=0–3, J' is a bond or O, S, S=O, SO_2, NH, OC=O, C=O, NR^7, $CR^7R^{7'}$,

$R^2P{=}O$, $R^2P{=}S$, $R^2OP{=}O$, $R^2NHP{=}O$, $OP{=}OOR^2$, $OP{=}ONHR^2$, $OP{=}OR^2$, OSO_2, NHNH, $NHNR^6$, NR^6NH, N═N, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, or heterocyclo or substituted heterocyclo, and J" is $(CR^7R^{7'})n$ and n=0–3, where Y is not a bond;

W is $CR^7R^{7'}$—$CR^7R^{7'}$, $CR^7R^{7'}$—C═O, NR^9—$CR^7R^{7'}$, N═CR^8, N═N, NR^9—$NR^{9'}$, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, or aryl or substituted aryl, wherein, when W is not NR^9—$CR^7R^{7'}$, N═CR^8, N═N, NR^9—$NR^{9'}$, or heterocyclo or substituted heterocyclo, then J' must be O, S, S═O, SO_2; NH, NR^7, $OP{=}OOR^2$, $OP{=}ONHR^2$, OSO_2, NHNH, $NHNR^6$, NR^6 NH, or N═N;

Q_1 is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocycloalkyl or substituted heterocycloalkyl, arylalkyl or substituted arylalkyl, alkynyl or substituted alkynyl, aryl or substituted aryl, heterocyclo (e.g., heteroaryl) or substituted heterocyclo (e.g., substituted heteroaryl), halo, CN, $R^1OC{=}O$, $R^4C{=}O$, $R^5R^6NC{=}O$, $HOCR^7R^{7'}$, nitro, R^1OCH_2, R^1O, NH_2, or NR^4R^5;

Q_2 is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocycloalkyl or substituted heterocycloalkyl, arylalkyl or substituted arylalkyl, alkynyl or substituted alkynyl, aryl or substituted aryl, heterocyclo (e.g., heteroaryl) or substituted heterocyclo (e.g., substituted heteroaryl), halo, CN, $R^1OC{=}O$, $R^4C{=}O$, $R^5R^6NC{=}O$, $HOCR^7R^{7'}$, nitro, R^1OCH_2, R^1O, NH_2, or NR^4R^5;

L is a bond, $(CR^7R^{7'})n$, NH, NR^5 or $NR^5(CR^7R^{7'})n$, where n=0–3;

R^1 and $R^{1'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkyalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl;

R^2 is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl;

R^3 and $R^{3'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, halo, CN, hydroxylamine, hydroxamide, alkoxy or substituted alkoxy, amino, NR^1R^2, thiol, alkylthio or substituted alkylthio;

R^4 is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, $R^1C{=}O$, $R^1NHC{=}O$, or $SO_2NR^1R^{1'}$;

R^5 is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, $R^1C{=}O$, $R^1NHC{=}O$, SO_2R^1, or $SO_2NR^1R^{1'}$;

R^6 is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, CN, OH, OR^1, $R^1C{=}O$, $R^1NHC{=}O$, SO_2R^1, or $SO_2NR^1R^{1'}$;

R^7 and $R^{7'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, halo, CN, OR^1, nitro, hydroxylamine, hydroxylamide, amino, NHR^4, NR^2R^5, NOR^1, thiol, alkylthio or Substituted alkylthio, $R^1C{=}O$, $R^1(C{=}O)O$, $R^1OC{=}O$, $R^1NHC{=}O$, SOR^1, $PO_3R^1R^{1'}$, $R^1R^{7'}NC{=}O$, $C{=}OSR^1$, SO_2R^1, or $SO_2NR^1R^{1'}$;

R^8 and $R^{8'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkyalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, nitro, halo, CN, OR^1, amino, NHR^4, NR^2R^5, NOR^1, alkylthio or substituted alkylthio, $C{=}OSR^1$, $R^1OC{=}O$, $R^1C{=}O$, $R^1NHC{=}O$, $R^1R^{1'}NC{=}O$, $S{=}OR^1$, SO_2R^1, $PO_3R^1R^{1'}$, or $SO_2NR^1R^{1'}$;

R^9 and $R^{9'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl; cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, CN, OH, OR^1, $R^1C{=}O$, $R^1OC{=}O$, $R^1NHC{=}O$, or $SO_2NR^1R^{1'}$;

especially where the groups W and Y of this preferred subgenus are also within the definitions of W' and Y' of formula Ia, with the provisos (1) to (14) of said formula Ia where appropriate to this subgenus, and most preferably where (i) when Y' is —O— and W' is $CR^7R^{7'}$—$CR^7R^{7'}$, A_1 and A_2 are not simultaneously CH; and (ii) when L is a bond, G is not an unsubstituted phenyl group.

Another, more preferred subgenus of the compounds of the invention includes compounds of the formula I or salts

thereof wherein one or more, preferably all, of the following substituents are as defined below:

G is an aryl or heterocyclo (e.g., heteroaryl) group, where said group is mono- or polycyclic, and which is optionally substituted at one or more positions, preferably with hydrogen, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, halo, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, aryl or substituted aryl, heterocyclo or substituted heterocyclo, arylalkyl or substituted arylalkyl, heterocycloalkyl or substituted heterocycloalkyl, CN, $R^1C{=}O$, $R^1HNC{=}O$, $R^1R^2NC{=}O$, $HOCR^3R^{3'}$, nitro, R^1OCH_2, R^1O, NH_2, NR^4R^5, SO_2R^1, or $SO_2NR^1R^{1'}$;

Z_1 is O;

Z_2 is O;

A_1 is CR^7;

A_2 is CR^7;

Y is J—J'—J" where J is $(CR^7R^{7'})n$ and n=0–3, J' is a bond or O, S, S═O, SO_2, NH, NR^7, $CR^7R^{7'}$, $R^2P{=}O$, $R^2P{=}S$, $R^2OP{=}O$, $R^2NHP{=}O$, $OP{=}OOR^2$, $OP{=}ONHR^2$, $OP{=}OR^2$, OSO_2, NHNH, $NHNR^6$, NR^6NH, N═N, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, or heterocyclo or substituted heterocyclo, and J" is $(CR^7R^{7'})n$ and n=0–3, where Y is not a bond;

W is $CR^7R^{7'}$—$CR^7R^{7'}$—$CR^7R^{7'}$—C═O, NR^9—$CR^7R^{7'}$, N═CR^8, N═N, NR^9—$NR^{9'}$, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, or aryl or substituted aryl, wherein, when W is not NR^9—$CR^7R^{7'}$, N═CR^8, N═N, NR^9—$NR^{9'}$; or heterocyclo or substituted heterocycle, then J' must be O, S, S═O, SO_2, NH, NR^7, $OP{=}OOR^2$, $OP{=}ONHR^2$, OSO_2, NHNH, $NHNR^6$, NR^6NH, or N═N;

Q_1 is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocycloalkyl or substituted heterocycloalkyl, arylalkyl or substituted arylalkyl, alkynyl or substituted alkynyl, aryl or substituted aryl, heterocyclo (e.g., heteroaryl) or substituted heterocyclo (e.g., substituted heteroaryl), halo, CN, $R^4C{=}O$, $R^5R^6NC{=}O$, $HOCR^7R^{7'}$, nitro, R^1OCH_2, R^1O, NH_2, or NR^4R^5;

Q_2 is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocycloalkyl or substituted heterocycloalkyl, arylalkyl or substituted arylalkyl, alkynyl or substituted alkynyl, aryl or substituted aryl, heterocyclo (e.g., heteroaryl) or substituted heterocyclo (e.g., substituted heteroaryl), halo, CN, $R^4C{=}O$, $R^5R^6NC{=}O$, $HOCR^7R^{7'}$, nitro, R^1OCH_2, R^1O, NH_2, or NR^4R^5;

L is a bond;

R^1 and $R^{1'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkyalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl;

R^2 is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl;

R^3 and $R^{3'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, halo, CN, alkoxy or substituted alkoxy, amino, NR^1R^2, alkylthio or substituted alkylthio;

R^4 is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, $R^1C{=}O$, $R^1NHC{=}O$, or $SO_2NR^1R^{1'}$;

R^5 is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, $R^1C{=}O$, $R^1NHC{=}O$, SO_2R^1, or $SO_2NR^1R^{1'}$;

R^6 is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, CN, OH, OR^1, $R^1C{=}O$, $R^1NHC{=}O$, SO_2R^1, or $SO_2NR^1R^{1'}$;

R^7 and $R^{7'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, halo, CN, OR^1, nitro, amino, NHR^4, NR^2R^5, alkylthio or substituted alkylthio, $R^1C{=}O$, $R^1(C{=}O)O$, R1NHC═O, SO_2R^1, $R^1R^{1'}NC{=}O$, or $SO_2NR^1R^{1'}$;

R^8 and $R^{8'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkyalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, nitro, halo, CN, OR^1, amino, NHR^4, NR^2R^5, alkylthio or substituted alkylthio, $R^1C{=}O$, $R^1NHC{=}O$, $R^1R^{1'}NC{=}O$, SO_2R^1, or $SO_2NR^1R^{1'}$; and

R^9 and $R^{9'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or

substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, CN, OH, OR^1, $R^1C{=}O$, $R^1NHC{=}O$, or $SO_2NR^1R^{1'}$;

especially where the groups W and Y of this preferred subgenus are also within the definitions of W' and Y' of formula Ia, with the provisos (1) to (14) of said formula Ia where appropriate to this subgenus, and most preferably where (i) when Y' is —O— and W' is $CR^7R^{7'}$—$CR^7R^{7'}$, A_1 and A_2 are not simultaneously CH; and (ii) when L is a bond, G is not an unsubstituted phenyl group.

A particularly preferred subgenus of the compounds of the invention includes compounds of the formula I or salts thereof wherein one or more, preferably all, of the substituents are as defined below:

G is an aryl (especially, phenyl or naphthyl) or heterocyclo (especially those heterocyclo groups G of the compounds of the Examples herein) group, where said group is mono- or polycyclic, and which is optionally substituted at one or more positions, preferably with substituents as exemplified in any of the compounds of the Examples herein;

L is a bond, $(CR^7R^{7'})n$ (where n is 1 and R^7 and $R^{7'}$ are each independently H, alkyl or substituted alkyl), or —CH2-NH—;

A_1 and A_2 are each independently CR^7 where R^7 (i) is hydrogen, alkyl or substituted alkyl, arylalkyl or substituted arylalkyl, alkenyl or substituted alkenyl (for example, alkenyl substituted with aryl (especially, phenyl or naphthyl) or substituted aryl, or alkenyl substituted with heterocyclo or substituted heterocyclo), aryl or substituted aryl, heterocyclo or substituted heterocyclo, heterocycloalkyl or substituted heterocycloalkyl, where, for each, preferred substituents are one or more groups selected from V^1 (especially A_1 and A_2, groups of the formula CR^7 where R^7 for each of A_1 and/or A_3 is independently selected from unsubstituted C_{1-4} alkyl, or C_{1-4} alkyl which alkyl is substituted by one or more groups V^1), or (ii) forms, together with R^7 of a group W (especially where W is $CR^7R^{7'}$—$CR^7R^{7'}$), a heterocyclic ring;

V^1 is OH, CN, halo, —O-aryl, —O-substituted aryl, —O-heterocyclo (e.g., —O-(optionally substituted pyridinyl) or —O-(optionally substituted pyrimidinyl)), —O-substituted heterocyclo, —O—CO-alkyl, —O—CO-substituted alkyl, —O-(alkylsilyl), —O-arylalkyl, —O-substituted arylalkyl, —O—CO-alkyl, —O—CO-substituted alkyl, —O—CO-arylalkyl, —O—CO-substituted arylalkyl, —O—CO-aryl, —O—CO-substituted aryl, —O—CO-heterocyclo, —O—CO-substituted heterocyclo, —S-(optionally substituted aryl)-NH—CO-(optionally substituted alkyl), —SO-(optionally substituted aryl)-NH—CO-(optionally substituted alkyl), —SO2-(optionally substituted aryl)-NH—CO-(optionally substituted alkyl), —NH—SO2-aryl, —NH—SO2-substituted aryl, —NH—CO—O-(optionally substituted arylalkyl), —NH—CO—O-alkyl, —NH—CO—O-substituted alkyl, —NH—CO-alkyl, —NH—CO-substituted alkyl, —NH—CO-aryl, —NH—CO-substituted aryl, —NH—CO-(optionally substituted arylalkyl), —NH—CO-(optionally substituted alkyl)-O-(optionally substituted aryl), —N(optionally substituted alkyl)(optionally substituted aryl), —N(optionally substituted alkyl)(optionally substituted arylalkyl), —COH, —COOH, —CO—O-alkyl, —CO—O-substituted alkyl, —CO—O-optionally substituted arylalkyl, —CO-aryl, —CO-substituted aryl, —O—CO—NH-aryl, —O—CO—NH-substituted aryl, —CO—NH-aryl, —CO—NH-substituted aryl, —CO—NH-arylalkyl, —CO—NH-substituted arylalkyl, —O-(optionally substituted aryl)-NH—CO-(optionally substituted alkyl);

Y is —O—, —SO—, —$N(V^2)$—, —CH_2—$N(V^2)$—, —CO—N(alkyl)-, —CH_2—S—; —CH_2—SO_2—;

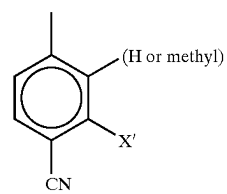
V^2 is hydrogen, alkyl, arylalkyl, —CO-alkyl, —CO—O-aryl, —CO—O-arylalkyl;

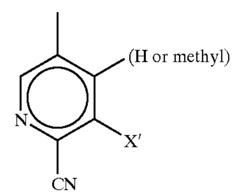
W is $CR^7R^{7'}$—$CR^7R^{7'}$ (where R^7 and $R^{7'}$ are each independently selected from H, OH, alkyl or substituted alkyl (such as hydroxyalkyl), or where R^7 forms a heterocyclic ring together with R^7 of A_1 or A_2), $CR^8{=}CR^{8'}$ (where R^8 and $R^{8'}$ are each independently selected from H, alkyl or substituted alkyl (such as hydroxyalkyl)), $CR^7R^{7'}$—C═O (where R^7 and $R^{7'}$ are each hydrogen, or where R^7 forms a heterocyclic ring together with R^7 of A_1 or A_2), $N{=}CR^8$ (where R^8 is alkyl), cycloalkyl or substituted cyclalkyl, or heterocyclo or substituted heterocyclo;

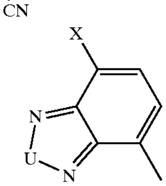
Z_1 and Z_2 are O; and

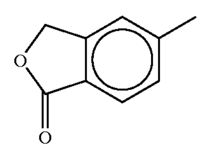
Q_1 and Q_2 are H.

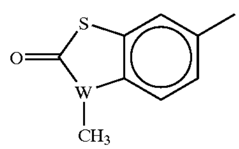
Preferred G—L groups are optionally substituted phenyl, optionally substituted naphthyl and optionally substituted fused bicyclic heterocyclic groups such as optionally substituted benzo-fused heterocyclic groups (e.g., bonded to the remainder of the molecule through the benzene portion), especially such groups wherein the heterocyclic ring bonded to benzene has 5 members exemplified by benzoxazole, benzothiazole, benzothiadiazole, benzoxadiazole or benzothiophene, for example:

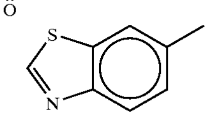


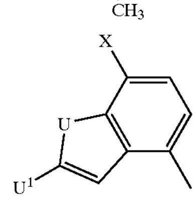


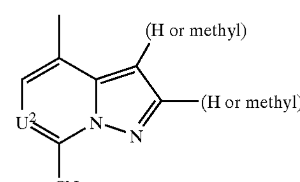


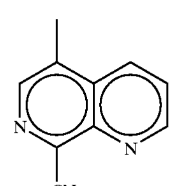


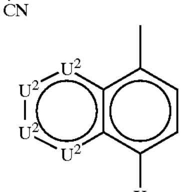












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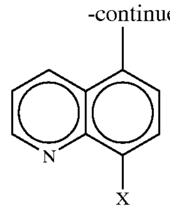
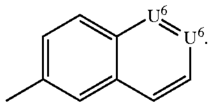
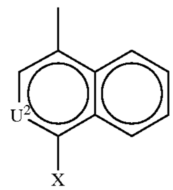
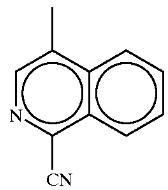
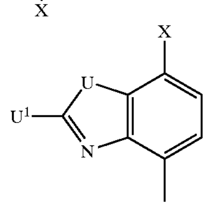
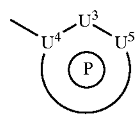
(e.g.,  where X is OH or CN) or

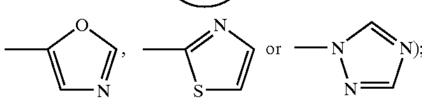
 

(e.g., ) or 

where

X=halo (especially F), OH, CN, NO_2 or



(e.g., );

X'=halo (especially Cl, F, or I), CH_3, CF_3, CN or OCH_3;

U is O or S (where S can optionally be oxygenated, e.g., to SO);

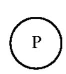
U^1 is CH_3 or CF_3;

each U^2 is independently N, CH or CF;

U^3 is N, O or S;

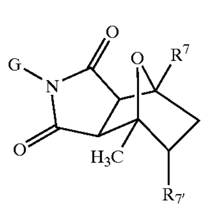
U^4 and U^5, together with the atoms to which they are bonded, form an optionally substituted 5-membered heterocyclic ring which can be partially unsaturated or aromatic and which contains 1 to 3 ring heteroatoms;

each U^6 is independently CH or N; and



denotes optional double bond(s) within the ring formed by U^3, U^4 and U^5.

An especially preferred subgenus includes compounds of the formula I having the following structure, or salts thereof:



where G is an optionally substituted phenyl, naphthyl or benzo-fused bicyclic heterocyclic group, R^7 is CH_3 or C_{1-4}alkyl substituted by V^1, and one $R^{7'}$ is H or hydroxyl and the other is H.

Compounds where $R^{7'}$ is hydroxyl can provide enhanced water solubility and metabolic stability, relative to the corresponding compounds where $R^{7'}$ is H, in addition to having good permeability and high systemic blood levels. These hydroxyl-bearing compounds can be obtained in vivo by metabolism of the corresponding compound where $R^{7'}$ is H, as well as by synthetic preparative methods such as those described herein.

Use and Utility

The compounds of the present invention modulate the function of nuclear hormone receptors (NHR), and include compounds which are, for example, agonists, partial agonists, antagonists or partial antagonists of the androgen receptor (AR), the estrogen receptor (ER), the progesterone receptor (PR), the glucocorticoid receptor (GR), the mineralocorticoid receptor (MR), the steroid and xenobiotic receptor (SXR), other steroid binding NHR's, the Orphan receptors or other NHR's. Selective modulation of one such NHR relative to others within the NHR family is preferred. "Modulation" includes, for example, activation (e.g., agonist activity such as selective androgen receptor agonist activity) or inhibition (e.g., antagonist activity).

The present compounds are thus useful in the treatment of NHR-associated conditions. A "NHR-associated condition", as used herein, denotes a condition or disorder which can be treated by modulating the function of a NHR in a subject, wherein treatment comprises prevention (e.g., prophylactic treatment), partial alleviation or cure of the condition or disorder. Modulation may occur locally, for example, within certain tissues of the subject, or more extensively throughout a subject being treated for such a condition disorder.

The compounds of the present invention are useful for the treatment of a variety of conditions and disorders including, but not limited to, those described following:

Compounds of formula I can be applied as agonists, partial agonists, antagonists, or partial antagonists of the estrogen receptor, preferably selectively to that receptor, in an array of medical conditions which involve modulation of the estrogen receptor pathway. Applications of said compounds include but are not limited to: osteoporosis, hot flushes, vaginal dryness, prostate cancer, breast cancer, endometrial cancer, cancers expressing the estrogen receptor such as the aforementioned cancers and others, contraception, pregnancy termination, menopause, amennoreahea, and dysmennoreahea.

Compounds of formula I can be applied as agonists, partial agonists, antagonists or partial antagonists of the progesterone receptor, preferably selectively to that receptor, in an array of medical conditions which involve modulation of the progesterone receptor pathway. Applications of said compounds include but are not limited to: breast cancer, other cancers containing the progesterone receptor, endometriosis, cachexia, contraception, menopause, cyclesynchrony, meniginoma, dysmennoreahea, fibroids, pregnancy termination, labor induction and osteoporosis.

Compounds of formula I can be applied as agonists, partial agonists, antagonists or partial antagonists of the glucocorticoid receptor, preferably selectively to that receptor, in an array of medical conditions which involve modulation of the glucocorticoid receptor pathway. Applications of said compounds include but are not limited to:

inflammatory diseases, autoimmune diseases, prostate cancer, breast cancer, Alzheimer's disease, psychotic disorders, drug dependence, non-insulin dependent Diabetes Mellitus, and as dopamine receptor blocking agents or otherwise as agents for the treatment of dopamine receptor mediated disorders.

Compounds of formula I can be applied as agonists, partial agonists, antagonists or partial antagonists of the mineralocorticoid receptor, preferably selectively to that receptor, in an array of medical conditions which involve modulation of the mineralocorticoid receptor pathway. Applications of said compounds include but are not limited to: drug withdrawal syndrome and inflammatory diseases.

Compounds of formula I can be applied as agonists, partial agonists, antagonists or partial antagonists of the aldosterone receptor, preferably selectively to that receptor, in an array of medical conditions which involve modulation of the aldosterone receptor pathway. One application of said compounds includes but is not limited to: congestive heart failure.

Compounds of formula I can be applied as agonists, partial agonists, antagonists or partial antagonists of the androgen receptor, preferably selectively to that receptor, in an array of medical conditions which involve modulation of the androgen receptor pathway. Applications of said compounds include but are not limited to: hirsutism, acne, seborrhea, Alzheimer's disease, androgenic alopecia, hypogonadism, hyperpilosity, benign prostate hypertrophia, adenomas and neoplasies of the prostate (such as advanced metastatic prostate cancer), treatment of benign or malignant tumor cells containing the androgen receptor such as is the case for breast, brain, skin, ovarian, bladder, lymphatic, liver and kidney cancers, pancreatic cancers modulation of VCAM expression and applications therein for the treatment of heart disease, inflammation and immune modulations, modulation of VEGF expression and the applications therein for use as antiangiogenic agents, osteoporosis, suppressing spermatogenesis, libido, cachexia, endometriosis, polycystic ovary syndrome, anorexia, androgen supplement for age related decreased testosterone levels in men, male menopause, male hormone replacement, male and female sexual dysfunction, and inhibition of muscular atrophy in ambulatory patients. For example, pan AR modulation is contemplated, with prostate selective AR modulation ("SARM") being particularly preferred, such as for the treatment of early stage prostate cancers.

Compounds of formula I can be applied as (preferably, selective) antagonists of the mutated androgen receptor, for example, found in many tumor lines. Examples of such mutants are those found in representative prostate tumor cell lines such as LNCap, (T877A mutation, Biophys. Acta, 187, 1052 (1990)), PCa2b, (L701H & T877A mutations, J. Urol., 162, 2192 (1999)) and CWR22, (H874Y mutation, Mol. Endo., 11, 450 (1997)). Applications of said compounds include but are not limited to: adenomas and neoplasies of the prostate, breast cancer and endometrial cancer.

Compounds of formula I can be applied as agonists, partial agonists, antagonists or partial antagonists of the steroid and xenobiotic receptor, preferably selectively to that receptor, in an array of medical conditions which involve modulation of the steroid and xenobiotic receptor pathway. Applications of said compounds include but are not limited to: treatment of disregulation of cholesterol homeostasis, attenuation of metabolism of pharmaceutical agents by co-administration of an agent (compound of the present invention) which modulates the P450 regulator effects of SXR.

Along with the aforementioned NHR, there also exist a number of NHR for which the activating or deactivating ligands may not be characterized. These proteins are classified as NHR due to strong sequence homology to other NHR, and are known as the Orphan receptors. Because the Orphan receptors demonstrate strong sequence homology to other NHR, compounds of formula I include those which serve as modulators of the function of the Orphan NHR. Orphan receptors which are modulated by NHR modulators such as compounds within the scope of formula I are exemplified, but not limited to, those listed in Table 1. Exemplary therapeutic applications of modulators of said Orphan receptors are also listed in Table 1, but are not limited to the examples therein.

Table 1. Exemplary Orphan nuclear hormone receptors, form (M=monomeric, D=heterodimeric, H=homodimeric), tissue expression and target therapeutic applications. (CNS=central nervous system)

TABLE 1

Exemplary Orphan nuclear hormone receptors, form (M = monomeric, D = heterodimeric, H = homodimeric), tissue expression and target therapeutic applications. (CNS = central nervous system)

Receptor	Form	Tissue Expression	Target Therapeutic Application
NURR1	M/D	Dopaminergic Neurons	Parkinson's Disease
RZRβ	M	Brain (Pituitary), Muscle	Sleep Disorders
RORα	M	Cerebellum, Purkinje Cells	Arthritis, Cerebellar Ataxia
NOR-1	M	Brain, Muscle, Heart, Adrenal, Thymus	CNS Disorders, Cancer
NGFI-Bβ	M/D	Brain	CNS Disorders
COUP-Tfα	H	Brain	CNS Disorders
COUP-TFβ	H	Brain	CNS Disorders
COUP-TFγχ	H	Brain	CNS Disorders
Nur77	H	Brain, Thymus, Adrenals	CNS Disorders
Rev-ErbAα	H	Muscle, Brain (Ubiquitous)	Obesity
HNF4α	H	Liver, Kidney, Intestine	Diabetes
SF-1	M	Gonads, Pituitary	Metabolic Disorders
LXRα,β	D	Kidney (Ubiquitous)	Metabolic Disorders
GCNF	M/H	Testes, Ovary	Infertility
ERRα,β	M	Placenta, Bone	Infertility, Osteoporosis
FXR	D	Liver, Kidney	Metabolic Disorders
CARα	H	Liver, Kidney	Metabolic Disorders
PXR	H	Liver, Intestine	Metabolic Disorders
COUP-TF2 (ARP1)	D	Testis	Oncology/angiogenesis
RORbeta	M	CNS, retina, pineal gland	Metabolic Disorders

The present invention thus provides methods for the treatment of NHR-associated conditions, comprising the step of administering to a subject in need thereof at least one compound of formula I in an amount effective therefor. Other therapeutic agents such as those described below may be employed with the inventive compounds in the present methods (for example, separately, or formulated together as a fixed dose). In the methods of the present invention, such other therapeutic agent(s) can be administered prior to, simultaneously with or following the administration of the compound(s) of the present invention.

The present invention also provides pharmaceutical compositions comprising at least one of the compounds of the formula I capable of treating a NHR-associated condition in an amount effective therefor, and a pharmaceutically acceptable carrier (vehicle or diluent). The compositions of the present invention can contain other therapeutic agents as described below, and can be formulated, for example, by employing conventional solid or liquid vehicles or diluents, as well as pharmaceutical additives of a type appropriate to

the mode of desired administration (for example, excipients, binders, preservatives, stabilizers, flavors, etc.) according to techniques such as those well known in the art of pharmaceutical formulation.

It should be noted that the compounds of the present invention are, without limitation as to their mechanism of action, useful in treating any of the conditions or disorders listed or described herein such as inflammatory diseases or cancers, or other proliferate diseases, and in compositions for treating such conditions or disorders. Such conditions and disorders include, without limitation, any of those described previously, as well as those described following such as: maintenance of muscle strength and function (e.g., in the elderly); reversal or prevention of frailty or age-related functional decline ("ARFD") in the elderly (e.g., sarcopenia); treatment of catabolic side effects of gliucocorticoids; prevention and/or treatment of reduced bone mass, density or growth (e.g., osteoporosis and osteopenia); treatment of chronic fatigue syndrome (CFS); chronic malagia; treatment of acute fatigue syndrome and muscle loss following elective surgery (e.g., post-surgical rehabilitation); acceleration of wound healing; accelerating bone fracture repair (such as accelerating the recovery of hip fracture patients); accelerating healing of complicated fractures, e.g. distraction osteogenesis; in joint replacement; prevention of post-surgical adhesion formation; acceleration of tooth repair or growth; maintenance of sensory function (e.g., hearing, sight, olefaction and taste); treatment of periodontal disease; treatment of wasting secondary to fractures and wasting in connection with chronic obstructive pulmonary disease (COPD), chronic liver disease, AIDS, weightlessness, cancer cachexia, burn and trauma recovery, chronic catabolic state (e.g., coma), eating disorders (e.g., anorexia) and chemotherapy; treatment of cardiomyopathy; treatment of thrombocytopenia; treatment of growth retardation in connection with Crohn's disease; treatment of short bowel syndrome; treatment of irritable bowel syndrome; treatment of inflammatory bowel disease, treatment of Crohn's disease and ulcerative colits; treatment of complications associated with transplantation; treatment of physiological short stature including growth hormone deficient children and short stature associated with chronic illness; treatment of obesity and growth retardation associated with obesity; treatment of anorexia (e.g., associated with cachexia or aging); treatment of hypercortisolism and Cushing's syndrome; Paget's disease; treatment of osteoarthritis; induction of pulsatile growth hormone release; treatment of osteochondrodysplasias; treatment of depression, nervousness, irritability and stress; treatment of reduced mental energy and low self-esteem (e.g., motivation/assertiveness); improvement of cognitive function (e.g., the treatment of dementia, including Alzheimer's disease and short term memory loss); treatment of catabolism in connection with pulmonary dysfunction and ventilator dependency; treatment of cardiac dysfunction (e.g., associated with valvular disease, myocardial infarction, cardiac hypertrophy or congestive heart failure); lowering blood pressure; protection against ventricular dysfunction or prevention of reperfusion events; treatment of adults in chronic dialysis; reversal or slowing of the catabolic state of aging; attenuation or reversal of protein catabolic responses following trauma (e.g., reversal of the catabolic state associated with surgery, congestive heart failure, cardiac myopathy, burns, cancer, COPD etc.); reducing cachexia and protein loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidioblastosis; treatment of immunosuppressed patients; treatment of wasting in connection with multiple sclerosis or other neurodegenerative disorders; promotion of myelin repair; maintenance of skin thickness; treatment of metabolic homeostasis and renal homeostasis (e.g., in the frail elderly); stimulation of osteoblasts, bone remodeling and cartilage growth; regulation of food intake; treatment of insulin resistance, including NIDDM, in mammals (e.g., humans); treatment of insulin resistance in the heart; improvement of sleep quality and correction of the relative hyposomatotropism of senescence due to high increase in REM sleep and a decrease in REM latency; treatment of hypothermia; treatment of congestive heart failure; treatment of lipodystrophy (e.g., in patients taking HIV or AIDS therapies such as protease inhibitors); treatment of muscular atrophy (e.g., due to physical inactivity, bed rest or reduced weight-bearing conditions); treatment of musculoskeletal impairment (e.g., in the elderly); improvement of the overall pulmonary function; treatment of sleep disorders; and the treatment of the catabolic state of prolonged critical illness; treatment of hirsutism, acne, seborrhea, androgenic alopecia, anemia, hyperpilosity, benign prostate hypertrophy, adenomas and neoplasies of the prostate (e.g., advanced metastatic prostate cancer) and malignant tumor cells containing the androgen receptor, such as is the case for breast, brain, skin, ovarian, bladder, lymphatic, liver and kidney cancers; cancers of the skin, pancreas, endometrium, lung and colon; osteosarcoma; hypercalcemia of malignancy; metastatic bone disease; treatment of spermatogenesis, endometriosis and polycystic ovary syndrome; conteracting preeclampsia, eclampsia of pregnancy and preterm labor; treatment of premenstrual syndrome; treatment of vaginal dryness; age related decreased testosterone levels in men, male menopause, hypogonadism, male hormone replacement, male and female sexual dysfunction (e.g., erectile dysfunction, decreased sex drive, sexual well-being, decreased libido), male and female contraception, hair loss, Reaven's Syndrome and the enhancement of bone and muscle performance/strength; and the conditions, diseases, and maladies collectively referenced to as "Syndrome X" or Metabolic Syndrome as detailed in Johannsson *J. Clin. Endocrinol. Metab.,* 82, 727–34 (1997).

The present compounds have therapeutic utility in the modulation of immune cell activation/proliferation, e.g., as competitive inhibitors of intercellular ligand/receptor binding reactions involving CAMs (Cellular Adhesion Molecules) and Leukointegrins. For example, the present compounds modulate LFA-ICAM 1, and are particularly useful as LFA-ICAM 1 antagonists, and in the treatment of all conditions associated with LFA-ICAM 1 such as immunological disorders. Preferred utilities for the present compounds include, but are not limited to: inflammatory conditions such as those resulting from a response of the non-specific immune system in a mammal (e.g., adult respiratory distress syndrome, shock, oxygen toxicity, multiple organ injury syndrome secondary to septicemia, multiple organ injury syndrome secondary to trauma, reperfusion injury of tissue due to cardiopulmonary bypass, myocardial infarction or use with thrombolysis agents, acute glomerulonephritis, vasculitis, reactive arthritis, dermatosis with acute inflammatory components, stroke, thermal injury, hemodialysis, leukapheresis, ulcerative colitis, necrotizing enterocolitis and granulocyte transfusion associated syndrome) and conditions resulting from a response of the specific immune system in a mammal (e.g., psoriasis, organ/tissue transplant rejection, graft vs. host reactions and autoimmune diseases including Raynaud's syndrome, autoimmune thyroiditis, dermatitis, multiple sclerosis, rheumatoid arthritis, insulin-

dependent diabetes mellitus, uveitis, inflammatory bowel disease including Crohn's disease and ulcerative colitis, and systemic lupus erythematosus). The present compounds can be used in treating asthma or as an adjunct to minimize toxicity with cytokine therapy in the treatment of cancers. The present compounds can be employed in the treatment of all diseases currently treatable through steroid therapy. The present compounds may be employed for the treatment of these and other disorders alone or with other immunosuppressive or antiinflammatory agents. In accordance with the invention, a compound of the formula I can be administered prior to the onset of inflammation (so as to suppress an anticipated inflammation) or after the initiation of inflammation. When provided prophylactically, the immunosupressive compound(s) are preferably provided in advance of any inflammatory response or symptom (for example, prior to, at, or shortly after the time of an organ or tissue transplant but in advance of any symptoms or organ rejection). The prophylactic administration of a compound of the formula I prevents or attenuates any subsequent inflammatory response (such as, for example, rejection of a transplanted organ or tissue, etc.) Administration of a compound of the formula I attenuates any actual inflammation (such as, for example, the rejection of a transplanted organ or tissue).

The compounds of the formula I can be administered for any of the uses described herein by any suitable means, for example, orally, such as in the form of tablets, capsules, granules or powders; sublingually; bucally; parenterally, such as by subcutaneous, intravenous, intramuscular, or intrasternal injection or infusion techniques (e.g., as sterile injectable aqueous or non-aqueous solutions or suspensions); nasally, including administration to the nasal membranes, such as by inhalation spray; topically, such as in the form of a cream or ointment; or rectally such as in the form of suppositories; in dosage unit formulations containing non-toxic, pharmaceutically acceptable vehicles or diluents. The present compounds can, for example, be administered in a form suitable for immediate release or extended release. Immediate release or extended release can be achieved by the use of suitable pharmaceutical compositions comprising the present compounds, or, particularly in the case of extended release, by the use of devices such as subcutaneous implants or osmotic pumps. The present compounds can also be administered liposomally.

Exemplary compositions for oral administration include suspensions which can contain, for example, microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners or flavoring agents such as those known in the art; and immediate release tablets which can contain, for example, microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and/or lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants such as those known in the art. The compounds of formula I can also be delivered through the oral cavity by sublingual and/or buccal administration. Molded tablets, compressed tablets or freeze-dried tablets are exemplary forms which may be used. Exemplary compositions include those formulating the present compound(s) with fast dissolving diluents such as mannitol, lactose, sucrose and/or cyclodextrins. Also included in such formulations may be high molecular weight excipients such as celluloses (avicel) or polyethylene glycols (PEG). Such formulations can also include an excipient to aid mucosal adhesion such as hydroxy propyl cellulose (HPC), hydroxy propyl methyl cellulose (HPMC), sodium carboxy methyl cellulose (SCMC), maleic anhydride copolymer (e.g., Gantrez), and agents to control release such as polyacrylic copolymer (e.g. Carbopol 934). Lubricants, glidants, flavors, coloring agents and stabilizers may also be added for ease of fabrication and use.

Exemplary compositions for nasal aerosol or inhalation administration include solutions in saline which can contain, for example, benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, and/or other solubilizing or dispersing agents such as those known in the art.

Exemplary compositions for parenteral administration include injectable solutions or suspensions which can contain, for example, suitable non-toxic, parenterally acceptable diluents or solvents, such as mannitol, 1,3-butanediol, water, Ringer's solution, an isotonic sodium chloride solution, or other suitable dispersing or wetting and suspending agents, including synthetic mono- or diglycerides, and fatty acids, including oleic acid, or Cremaphor.

Exemplary compositions for rectal administration include suppositories which can contain, for example, a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperatures, but liquify and/or dissolve in the rectal cavity to release the drug.

Exemplary compositions for topical administration include a topical carrier such as Plastibase (mineral oil gelled with polyethylene).

The effective amount of a compound of the present invention can be determined by one of ordinary skill in the art, and includes exemplary dosage amounts for an adult human of from about 1 to 100 (for example, 15 or lower, especially 1 to 3 or less) mg/kg of body weight of active compound per day, which can be administered in a single dose or in the form of individual divided doses, such as from 1 to 4 times per day. It will be understood that the specific dose level and frequency of dosage for any particular subject can be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the species, age, body weight, general health, sex and diet of the subject, the mode and time of administration, rate of excretion, drug combination, and severity of the particular condition. Preferred subjects for treatment include animals, most preferably mammalian species such as humans, and domestic animals such as dogs, cats and the like, subject to NHR-associated conditions.

As mentioned above, the compounds of the present invention can be employed alone or in combination with each other and/or other suitable therapeutic agents useful in the treatment of NHR-associated conditions, e.g., an antibiotic or other pharmaceutically active material.

For example, the compounds of the present invention can be combined with growth promoting agents, such as, but not limited to, TRH, diethylstilbesterol, theophylline, enkephalins, E series prostaglandins, compounds disclosed in U.S. Pat. No. 3,239,345, e.g., zeranol, and compounds disclosed in U.S. Pat. No. 4,036,979, e.g., sulbenox or peptides disclosed in U.S. Pat. No. 4,411,890.

The compounds of the invention can also be used in combination with growth hormone secretagogues such as GHRP-6, GHRP-1 (as described in U.S. Pat. No. 4,411,890 and publications WO 89/07110 and WO 89/07111), GHRP-2 (as described in WO 93/04081), NN703 (Novo Nordisk), LY444711 (Lilly), MK-677 (Merck), CP424391 (Pfizer) and B-HT920, or with growth hormone releasing factor and its analogs or growth hormone and its analogs or somatomedins

including IGF-1 and IGF-2, or with alpha-adrenergic agonists, such as clonidine or serotinin 5-HT_D agonists, such as sumatriptan, or agents which inhibit somatostatin or its release, such as physostigmine and pyridostigmine. A still further use of the disclosed compounds of the invention is in combination with parathyroid hormone, PTH(1-34) or bisphosphonates, such as MK-217 (alendronate).

A still further use of the compounds of the invention is in combination with estrogen, testosterone, a selective estrogen receptor modulator, such as tamoxifen or raloxifene, or other androgen receptor modulators, such as those disclosed in Edwards, J. P. et al., *Bio. Med. Chem. Let.,* 9, 1003–1008 (1999) and Hamann, L. G. et al., *J. Med. Chem.,* 42, 210–212 (1999).

A further use of the compounds of this invention is in combination with progesterone receptor agonists ("PRA"), such as levonorgestrel, medroxyprogesterone acetate (MPA).

The compounds of the present invention can be employed alone or in combination with each other and/or other modulators of nuclear hormone receptors or other suitable therapeutic agents useful in the treatment of the aforementioned disorders including: anti-diabetic agents; anti-osteoporosis agents; anti-obesity agents; anti-inflammatory agents; anti-anxiety agents; anti-depressants; anti-hypertensive agents; anti-platelet agents; anti-thrombotic and thrombolytic agents; cardiac glycosides; cholesterol/lipid lowering agents; mineralocorticoid receptor antagonists; phospodiesterase inhibitors; protein tyrosine kinase inhibitors; thyroid mimetics (including thyroid receptor agonists); anabolic agents; HIV or AIDS therapies; therapies useful in the treatment of Alzheimer's disease and other cognitive disorders; therapies useful in the treatment of sleeping disorders; anti-proliferative agents; and anti-tumor agents.

Examples of suitable anti-diabetic agents for use in combination with the compounds of the present invention include biguanides (e.g., metformin), glucosidase inhibitors (e.g,. acarbose), insulins (including insulin secretagogues or insulin sensitizers), meglitinides (e.g., repaglinide), sulfonylureas. (e.g., glimepiride, glyburide and glipizide), biguanide/glyburide combinations (e.g., Glucovance®), thiazolidinediones (e.g., troglitazone, rosiglitazone and pioglitazone), PPAR-alpha agonists, PPAR-gamma agonists, PPAR alpha/gamma dual agonists, SGLT2 inhibitors, glycogen phosphorylase inhibitors, inhibitors of fatty acid binding protein (aP2) such as those disclosed in U.S. Ser. No. 09/519,079 filed Mar. 6, 2000, glucagon-like peptide-1 (GLP-1), and dipeptidyl peptidase IV (DP4) inhibitors.

Examples of suitable anti-osteoporosis agents for use in combination with the compounds of the present invention include alendronate, risedronate, PTH, PTH fragment, raloxifene, calcitonin, steroidal or non-steroidal progesterone receptor agonists, RANK ligand antagonists, calcium sensing receptor antagonists, TRAP inhibitors, selective estrogen receptor modulators (SERM), estrogen and AP-1 inhibitors.

Examples of suitable anti-obesity agents for use in combination with the compounds of the present invention include aP2 inhibitors, such as those disclosed in U.S. Ser. No. 09/519,079 filed Mar. 6, 2000, PPAR gamma antagonists, PPAR delta agonists, beta 3 adrenergic agonists, such as AJ9677 (Takeda/Dainippon), L750355 (Merck), or CP331648 (Pfizer) or other known beta 3 agonists as disclosed in U.S. Pat. Nos. 5,541,204, 5,770,615, 5,491,134, 5,776,983 and 5,488,064, a lipase inhibitor, such as orlistat or ATL-962 (Alizyme), a serotonin (and dopamine) reuptake inhibitor, such as sibutramine, topiramate (Johnson & Johnson) or axokine (Regeneron), a thyroid receptor beta drug, such as a thyroid receptor ligand as disclosed in WO 97/21993 (U. Cal SF), WO 99/00353 (KaroBio) and GB98/284425 (KaroBio), and/or an anorectic agent, such as dexamphetamine, phentermine, phenylpropanolamine or mazindol.

Examples of suitable anti-inflammatory agents for use in combination with the compounds of the present invention include prednisone, dexamethasone, Enbrel®, cyclooxygenase inhibitors (i.e., COX-1 and/or COX-2 inhibitors such as NSAIDs, aspirin, indomethacin, ibuprofen, piroxicam, Naproxen®, Celebrex®, Vioxx®), CTLA4-Ig agonists/antagonists, CD40 ligand antagonists, IMPDH inhibitors, such as mycophenolate (CellCept®) integrin antagonists, alpha-4 beta-7 integrin antagonists, cell adhesion inhibitors, interferon gamma antagonists, ICAM-1, tumor necrosis factor (TNF) antagonists (e.g., infliximab, OR1384), prostaglandin synthesis inhibitors, budesonide, clofazimine, CNI-1493, CD4 antagonists (e.g., priliximab), p38 mitogen-activated protein kinase inhibitors, protein tyrosine kinase (PTK) inhibitors, IKK inhibitors, and therapies for the treatment of irritable bowel syndrome (e.g., Zelmac® and Maxi-K® openers such as those disclosed in U.S. Pat. No. 6,184,231 B1).

Example of suitable anti-anxiety agents for use in combination with the compounds of the present invention include diazepam, lorazepam, buspirone, oxazepam, and hydroxyzine pamoate.

Examples of suitable anti-depressants for use in combination with the compounds of the present invention include citalopram, fluoxetine, nefazodone, sertraline, and paroxetine.

Examples of suitable anti-hypertensive agents for use in combination with the compounds of the present invention include beta adrenergic blockers, calcium channel blockers (L-type and T-type; e.g. diltiazem, verapamil, nifedipine, amlodipine and mybefradil), diuretics (e.g., chlorothiazide, hydrochlorothiazide, flumethiazide, hydroflumethiazide, bendroflumethiazide, methylchlorothiazide, trichloromethiazide, polythiazide, benzthiazide, ethacrynic acid tricrynafen, chlorthalidone, furosemide, musolimine, bumetanide, triamtrenene, amiloride, spironolactone), renin inhibitors, ACE inhibitors (e.g., captopril, zofenopril, fosinopril, enalapril, ceranopril, cilazopril, delapril, pentopril, quinapril, ramipril, lisinopril), AT-1 receptor antagonists (e.g., losartan, irbesartan, valsartan), ET receptor antagonists (e.g., sitaxsentan, atrsentan and compounds disclosed in U.S. Pat. Nos. 5,612,359 and 6,043,265), Dual ET/AII antagonist (e.g., compounds disclosed in WO 00/01389), neutral endopeptidase (NEP) inhibitors, vasopepsidase inhibitors (dual NEP-ACE inhibitors) (e.g., omapatrilat and gemopatrilat), and nitrates.

Examples of suitable anti-platelet agents for use in combination with the compounds of the present invention include GPIIb/IIIa blockers (e.g., abciximab, eptifibatide, tirofiban), P2Y12 antagonists (e.g., clopidogrel, ticlopidine, CS-747), thromboxane receptor antagonists (e.g., ifetroban), aspirin, and PDE-III inhibitors (e.g., dipyridamole) with or without aspirin.

Examples of suitable cardiac glycosides for use in combination with the compounds of the present invention include digitalis and ouabain.

Examples of suitable cholesterol/lipid lowering agents for use in combination with the compounds of the present invention include HMG-CoA reductase inhibitors (e.g., pravastatin, lovastatin, atorvastatin, simvastatin, NK-104 (a.k.a. itavastatin, or nisvastatin or nisbastatin) and ZD-4522

(a.k.a. rosuvastatin, or atavastatin or visastatin)), squalene synthetase inhibitors, fibrates, bile acid sequestrants, ACAT inhibitors, MTP inhibitors, lipooxygenase inhibitors, cholesterol absorption inhibitors, and cholesterol ester transfer protein inhibitors (e.g., CP-529414).

Examples of suitable mineralocorticoid receptor antagonists for use in combination with the compounds of the present invention include spironolactone and eplerinone.

Examples of suitable phospodiesterase inhibitors for use in combination with the compounds of the present invention include PDEIII inhibitors such as cilostazol, and PDE V inhibitors such as sildenafil.

Examples of suitable thyroid mimetics for use in combination with the compounds of the present invention include thyrotropin, polythyroid, KB-130015, and dronedarone.

Examples of suitable anabolic agents for use in combination with the compounds of the present invention include testosterone, TRH diethylstilbesterol, estrogens, β-agonists, theophylline, anabolic steroids, dehydroepiandrosterone, enkephalins, E-series prostagladins, retinoic acid and compounds as disclosed in U.S. Pat. No. 3,239,345, e.g., Zeranol®; U.S. Pat. No. 4,036,979, e.g., Sulbenox® or peptides as disclosed in U.S. Pat. No. 4,411,890.

Examples of suitable HIV or AIDS therapies for use in combination with the compounds of the present invention include indinavir sulfate, saquinavir, saquinavir mesylate, ritonavir, lamivudine, zidovudine, lamivudine/zidovudine combinations, zalcitabine, didanosine, stavudine, and megestrol acetate.

Examples of suitable therapies for treatment of Alzheimer's disease and cognitive disorders for use in combination with the compounds of the present invention include donepezil, tacrine, revastigmine, 5HT6, gamma secretase inhibitors, beta secretase inhibitors, SK channel blockers, Maxi-K blockers, and KCNQs blockers.

Examples of suitable therapies for treatment of sleeping disorders for use in combination with the compounds of the present invention include melatonin analogs, melatonin receptor antagonists, ML1B agonists, and GABA/NMDA receptor antagonists.

Examples of suitable anti-proliferative agents for use in combination with the compounds of the present invention include cyclosporin A, paclitaxel, FK 506, and adriamycin.

Examples of suitable anti-tumor agents for use in combination with the compounds of the present invention include paclitaxel, adriamycin, epothilones, cisplatin and carboplatin.

Compounds of the present invention can further be used in combination with nutritional supplements such as those described in U.S. Pat. No. 5,179,080, especially in combination with whey protein or casin, amino acids (such as leucine, branched amino acids and hydroxymethylbutyrate), triglycerides, vitamins (e.g., A, B6, B12, folate, C, D and E), minerals (e.g., selenium, magnesium, zinc, chromium, calcium and potassium), carnitine, lipoic acid, creatine, and coenzyme Q-10.

In addition, compounds of the present invention can be used in combination with therapeutic agents used in the treatment of sexual dysfunction, including but not limited to PDE5 inhibitors, such as sildenafil or IC-351; with an antiresorptive agent, hormone replacement therapies, vitamin D analogues, calcitonins, elemental calcium and calcium supplements, cathepsin K inhibitors, MMP inhibitors, vitronectin receptor antagonists, Src SH_2 antagonists, vacular —H^+-ATPase inhibitors, progesterone receptor agonists, ipriflavone, fluoride, RANK antagonists, PTH and its analogues and fragments, Tibolone, HMG-CoA reductase inhibitors, SERM's, p38 inhibitors, prostanoids, 17-beta hydroxysteroid dehydrogenase inhibitors and Src kinase inhibitors.

Compounds of the present invention can be used in combination with male contraceptives, such as nonoxynol 9 or therapeutic agents for the treatment of hair loss, such as minoxidil and finasteride or chemotherapeutic agents, such as with LHRH agonists.

For their preferred anticancer or antiangiogenic use, the compounds of the present invention can be administered either alone or in combination with other anti-cancer and cytotoxic agents and treatments useful in the treatment of cancer or other proliferative diseases, for example, where the second drug has the same or different mechanism of action than the present compounds of formula I. Examples of classes of anti-cancer and cytotoxic agents useful in combination with the present compounds include but are not limited to: alkylating agents such as nitrogen mustards, alkyl sulfonates, nitrosoureas, ethylenimines, and triazenes; EGFR inhibitors such as small molecule EGFR inhibitors, EGFR antibodies such as C225 (Erbitux); antimetabolites such as folate antagonists, purine analogues, and pyrimidine analogues; antibiotics such as anthracyclines, bleomycins, mitomycin, dactinomycin, and plicamycin; enzymes such as L-asparaginase; farnesyl-protein transferase inhibitors; 5α reductase inhibitors; inhibitors of 17β-hydroxy steroid dehydrogenase type 3 or type 1; hormonal agents such as glucocorticoids, estrogens/antiestrogens, androgens/antiandrogens, progestins, and luteinizing hormone-releasing hormone antagonists, octreotide acetate; microtubule-disruptor agents, such as ecteinascidins or their analogs and derivatives; microtubule-stabilizing agents such as taxanes, for example, paclitaxel (Taxol®), docetaxel (Taxotere®), and their analogs, and epothilones, such as epothilones A–F and their analogs; plant-derived products, such as vinca alkaloids, epipodophyllotoxins, taxanes; and topiosomerase inhibitors; prenyl-protein transferase inhibitors; and miscellaneous agents such as hydroxyurea, procarbazine, mitotane, hexamethylmelamine, platinum coordination complexes such as cisplatin and carboplatin; and other agents used as anti-cancer and cytotoxic agents such as biological response modifiers, growth factors; immune modulators and monoclonal antibodies. The compounds of the invention may also be used in conjunction with radiation therapy.

Representative examples of these classes of anti-cancer and cytotoxic agents include but are not limited to mechlorethamine hydrochloride, cyclophosphamide, chlorambucil, melphalan, ifosfamide, busulfan, carmustin, lomustine, semustine, streptozocin, thiotepa, dacarbazine, methotrexate, thioguanine, mercaptopurine, fludarabine, pentastatin, cladribin, cytarabine, fluorouracil, doxorubicin hydrochloride, daunorubicin, idarubicin, bleomycin sulfate, mitomycin C, actinomycin D, safracins, saframycins, quinocarcins, discodermolides, vincristine, vinblastine, vinorelbine tartrate, etoposide, etoposide phosphate, teniposide, paclitaxel, tamoxifen, estramustine, estramustine phosphate sodium, flutamide, buserelin, leuprolide, pteridines, diyneses, levamisole, aflacon, interferon, interleukins, aldesleukin, filgrastim, sargramostim, rituximab, BCG, tretinoin, irinotecan hydrochloride, betamethosone, gemcitabine hydrochloride, altretamine, and topoteca and any analogs or derivatives thereof.

Preferred member of these classes include, but are not limited to, paclitaxel, cisplatin, carboplatin, doxorubicin, carminomycin, daunorubicin, aminopterin, methotrexate, methopterin, mitomycin C, ecteinascidin 743, or

porfiromycin, 5-fluorouracil, 6-mercaptopurine, gemcitabine, cytosine arabinoside, podophyllotoxin or podophyllotoxin derivatives such as etoposide, etoposide phosphate or teniposide, melphalan, vinblastine, vincristine, leurosidine, vindesine and leurosine.

Examples of anticancer and other cytotoxic agents include the following: epothilone derivatives as found in German Patent No. 4138042.8; WO 97/19086, WO 98/22461, WO 98/25929, WO 98/38192, WO 99/01124, WO 99/02224, WO 99/02514, WO 99/03848, WO 99/07692, WO 99/27890, WO 99/28324, WO 99/43653, WO 99/54330, WO 99/54318, WO 99/54319, WO 99/65913, WO 99/67252, WO 99/67253 and WO 00/00485; cyclin dependent kinase inhibitors as found in WO 99/24416 (see also U.S. Pat. No. 6,040,321); and prenyl-protein transferase inhibitors as found in WO 97/30992 and WO 98/54966; and agents such as those described generically and specifically in U.S. Pat. No. 6,011,029 (the compounds of which U.S. patent can be employed together with any NHR modulators (including, but not limited to, those of present invention) such as AR modulators, ER modulators, with LHRH modulators, or with surgical castration, especially in the treatment of cancer).

The combinations of the present invention can also be formulated or co-administered with other therapeutic agents that are selected for their particular usefulness in administering therapies associated with the aforementioned conditions. For example, the compounds of the invention may be formulated with agents to prevent nausea, hypersensitivity and gastric irritation, such as antiemetics, and H_1 and H_2 antihistaminics.

As it pertains to the treatment of cancer, the compounds of this invention are most preferably used alone or in combination with anti-cancer treatments such as radiation therapy and/or with cytostatic and/or cytotoxic agents, such as, but not limited to, DNA interactive agents, such as cisplatin or doxorubicin; inhibitors of farnesyl protein transferase, such as those described in U.S. Pat. No. 6,011,029; topoisomerase II inhibitors, such as etoposide; topoisomerase I inhibitors, such as CPT-11 or topotecan; tubulin stabilizing agents, such as paclitaxel, docetaxel, other taxanes, or epothilones; hormonal agents, such as tamoxifen; thymidilate synthase inhibitors, such as 5-fluorouracil; antimetabolites, such as methoxtrexate; antiangiogenic agents, such as angiostatin, ZD6474, ZD6126 and comberstatin A2; kinase inhibitors, such as her2 specific antibodies, Iressa and CDK inhibitors; histone deacetylase inhibitors, such as CI-994 and MS-27-275. Such compounds may also be combined with agents which suppress the production of circulating testosterone such as LHRH agonists or antagonists or with surgical castration. Exemplary combination therapies (e.g., for the treatment of prostate cancer) for use with a compound of the present invention include an LHRH modulator or prednisone.

The present invention also contemplates kits, for example, for the treatment of prostate cancer, comprising a first container (such as a vial) containing a pharmaceutical formulation comprising a compound of the present invention, said compound optionally in a pharmaceutically acceptable carrier, and a second container (such as a vial) containing a pharmaceutical formulation comprising one or more agents (such as an LHRH modulator) to be used in combination with said compound of the present invention, said agent(s) optionally in a pharmaceutically acceptable carrier.

For example, known therapies for advanced metastatic prostate cancer include "complete androgen ablation therapy" wherein tumor growth is inhibited by controlling the supply of androgen to the prostate tissues via chemical castration (castration serves to inhibit the production of circulating testosterone (T) and dihydrotestosterone (DHT)) followed by the administration of androgen receptor (AR) antagonists (which inhibit the function T/DHT derived from the conversion of circulating androgen precursors to T/DHT by the prostate tissue). The compounds of the present invention can be employed as AR antagonists in complete ablation therapy, alone or in combination with other AR antagonists such as Flutamide, Casodex, Nilutamide, or Cyproterone acetate.

The present invention provides compounds which can be used to treat patients suffering from prostate cancer resistant to androgen receptor antagonists which are not within formula I of the invention (or salts thereof), such as bicalutimide. The invention thus further contemplates a method of treating prostate cancer resistant to an androgen receptor antagonist other than those of formula I or salts thereof, comprising the step of administering to a patient in need thereof a compound capable of reducing the growth rate of the tumor mass of said cancer in an amount effective therefor. The term "reducing the growth rate of said tumor mass" denotes reduction in the growth rate (including, of course, stabilization or reduction in size) of said tumor mass upon treatment relative to the growth rate upon treatment with said androgen receptor antagonist other than those of formula I or salts thereof. Compounds of the formula I and pharmaceutically acceptable salts thereof of the present invention are preferred such compounds.

The present invention also contemplates use of an antiestrogen and/or aromatase inhibitor in combination with a compound of the present invention, for example, to assist in mitigating side effects associated with antiandrogen therapy such as gynecomastia. Exemplary antiestrogen and/or aromatase inhibitors include anastrozole (Arimidex), tamoxifen citrate (Nolvadex), exemestane (Aromasin), toremifene citrate (Fareston), letrozole (Femara), raloxifene hydrochloride (Evista), Faslodex, or 923 (Wyeth Ayerst).

The compounds of the present invention may be employed adjuvant to surgery.

Another application of the present compounds is in combination with antibody therapy such as but not limited to antibody therapy against PSCA. An additional application is in concert with vaccine/immune modulating agents for the treatment of cancer.

Compounds of the present invention can be employed in accordance with the methods described in U.S. Provisional Patent Application Ser. No. 60/284,438, entitled "Selective Androgen Receptor Modulators and Methods for Their Identification, Design and Use" filed Apr. 18, 2001 by Mark E. Salvati et al. which Provisional Patent Application is incorporated herein by reference in its entirety (including, but not limited to, reference to all specific compounds within formula I of the present invention), and U.S. patent application Ser. No. 09/885,827, entitled "Selective Androgen Receptor Modulators and Methods for Their Identification, Design and Use" filed Jun. 20, 2001 by Mark E. Salvati et al. which Patent Application is incorporated herein by reference in its entirety (including, but not limited to, reference to all specific compounds within formula I of the present invention).

For racemates of compounds of the present invention, one enantiomer can, for example be a full AR antagonist while the other can be an AR antagonist in tumor tissue while having no activity or agonist activity in nontumor tissue containing the androgen receptor.

The above other therapeutic agents, when employed in combination with the compounds of the present invention,

can be used, for example, in those amounts indicated in the Physicians' Desk Reference (PDR) or as otherwise determined by one of ordinary skill in the art.

The following assays can be employed in ascertaining the activity of a compound as a NHR modulator. Preferred are those compounds with an activity greater than 20 μm for binding or transactivation in any of these assays. Various compounds of the present invention were determined to have AR modulator activity utilizing the transactivation assay, and standard AR binding assays as described following.

Transactivation Assays:

AR Specific Assay:

Compounds of the present invention were tested in transactivation assays of a transfected reporter construct and using the endogenous androgen receptor of the host cells. The transactivation assay provides a method for identifying functional agonists and partial agonists that mimic, or antagonists that inhibit, the effect of native hormones, in this case, dihydrotestosterone (DHT). This assay can be used to predict in vivo activity as there is a good correlation in both series of data. See, e.g. T. Berger et al., *J. Steroid Biochem. Molec. Biol.* 773 (1992), the disclosure of which is herein incorporated by reference.

For the transactivation assay a reporter plasmid is introduced by transfection (a procedure to induce cells to take foreign genes) into the respective cells. This reporter plasmid, comprising the cDNA for a reporter protein, such as secreted alkaline phosphatase (SEAP), controlled by prostate specific antigen (PSA) upstream sequences containing androgen response elements (AREs). This reporter plasmid functions as a reporter for the transcription-modulating activity of the AR. Thus, the reporter acts as a surrogate for the products (mRNA then protein) normally expressed by a gene under control of the AR and its native hormone. In order to detect antagonists, the transactivation assay is carried out in the presence of constant concentration of the natural AR hormone (DHT) known to induce a defined reporter signal. Increasing concentrations of a suspected antagonist will decrease the reporter signal (e.g., SEAP production). On the other hand, exposing the transfected cells to increasing concentrations of a suspected agonist will increase the production of the reporter signal.

For this assay, LNCaP and MDA 453 cells were obtained from the American Type Culture Collection (Rockville, Md.), and maintained in RPMI 1640 or DMEM medium supplemented with 10% fetal bovine serum (FBS; Gibco) respectively. The respective cells were transiently transfected by electroporation according to the optimized procedure described by Heiser, 130 Methods Mol. Biol., 117 (2000), with the pSEAP2/PSA540/Enhancer reporter plasmid. The reporter plasmid, was constructed as follows: commercial human placental genomic DNA was used to generate by Polymerase Cycle Reaction (PCR) a fragment containing the BglII site (position 5284) and the Hind III site at position 5831 of the human prostate specific antigen promoter (Accession # U37672), Schuur, et al., *J. Biol. Chem.,* 271 (12): 7043–51 (1996). This fragment was subcloned into the pSEAP2/basic (Clontech) previously digested with BglII and HindIII to generate the pSEAP2/PSA540 construct. Then a fragment bearing the fragment of human PSA upstream sequence between positions −5322 and −3873 was amplified by PCR from human placental genomic DNA. A XhoI and a BglII sites were introduced with the primers. The resulting fragment was subcloned into pSEAP2/PSA540 digested with XhoI and BglII respectively, to generate the pSEAP2/PSA540/Enhancer construct.

LNCaP and MDA 453 cells were collected in media containing 10% charcoal stripped FBS. Each cell suspension was distributed into two Gene Pulser Cuvetts (Bio-Rad) which then received 8 μg of the reporter construct, and electoporated using a Bio-Rad Gene Pulser at 210 volts and 960 μFaraday. Following the transfections the cells were washed and incubated with media containing charcoal stripped fetal bovine serum in the absence (blank) or presence (control) of 1 nM dihydrotestosterone (DHT; Sigma Chemical) and in the presence or absence of the standard anti-androgen bicalutamide or compounds of the present invention in concentrations ranging from 10−10 to 10−5 M (sample). Duplicates were used for each sample. The compound dilutions were performed on a Biomek 2000 laboratory workstation. After 48 hours, a fraction of the supernatant was assayed for SEAP activity using the Phospha-Light Chemiluminescent Reporter Gene Assay System (Tropix, Inc). Viability of the remaining cells was determined using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (MTS Assay, Promega). Briefly, a mix of a tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) and an electron coupling reagent (phenazine methosulfate; PMS) are added to the cells. MTS (Owen's reagent) is bioreduced by cells into a formazan that is soluble in tissue culture medium, and therefore its absorbance at 490 nm can be measured directly from 96 well assay plates without additional processing. The quantity of formazan product as measured by the amount of 490 nm absorbance is directly proportional to the number of living cells in culture. For each replicate the SEAP reading was normalized by the Abs490 value derived from the MTS assay. For the antagonist mode, the % Inhibition was calculated as:

% Inhibition=100×(1−[average control−average blank/average sample−average blank])

Data was plotted and the concentration of compound that inhibited 50% of the normalized SEAP was quantified (IC_{50}).

For the agonist mode % Control was referred as the effect of the tested compound compared to the maximal effect observed with the natural hormone, in this case DHT, and was calculated as:

% Control=100×average sample−average blank/average control−average blank

Data was plotted and the concentration of compound that activates to levels 50% of the normalized SEAP for the control was quantified (EC_{50}).

GR Specificity Assay:

The reporter plasmid utilized was comprised of the cDNA for the reporter SEAP protein, as described for the AR specific transactivation assay. Expression of the reporter SEAP protein was controlled by the mouse mammary tumor virus long terminal repeat (MMTV LTR) sequences that contains three hormone response elements (HREs) that can be regulated by both GR and PR see, e.g. G. Chalepakis et al., Cell, 53(3), 371 (1988). This plasmid was transfected into A549 cells, which expresses endogenous GR, to obtain a GR specific transactivation assay. A549 cells were obtained from the American Type Culture Collection (Rockville, Md.), and maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS; Gibco). Determination of the GR specific antagonist activity of the compounds of the present invention was identical to that described for the AR specific transactivation assay, except

that the DHT was replaced with 5 nM dexamethasone (Sigma Chemicals), a specific agonist for GR. Determination of the GR specific agonist activity of the compounds of the present invention was performed as described for the AR transactivation assay, wherein one measures the activation of the GR specific reporter system by the addition of a test compound, in the absence of a known GR specific agonists ligand.

PR Specific Assay:

The reporter plasmid utilized was comprised of the cDNA for the reporter SEAP protein, as described for the AR specific transactivation assay. Expression of the reporter SEAP protein was controlled by the mouse mammary tumor virus long terminal repeat (MMTV LTR) sequences that contains three hormone response elements (HREs) that can be regulated by both GR and PR. This plasmid was transfected into T47D, which expresses endogenous PR, to obtain a PR specific transactivation assay. T47D cells were obtained from the American Type Culture Collection (Rockville, Md.), and maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS; Gibco). Determination of the PR specific antagonist activity of the compounds of the present invention was identical to that described for the AR specific transactivation assay, except that the DHT was replaced with 1 nM Promegastone (NEN), a specific agonist for PR. Determination of the PR specific agonist activity of the compounds of the present invention was performed as described for the AR transactivation assay, wherein one measures the activation of the PR specific reporter system by the addition of a test compound, in the absence of a known PR specific agonists ligand.

AR Binding Assay:

For the whole cell binding assay, human LNCaP cells (T877A mutant AR) or MDA 453 (wild type AR) in 96-well microtiter plates containing RPMI 1640 or DMEM supplemented with 10% charcoal stripped CA-FBS (Cocaleco Biologicals) respectively, were incubated at 37° C. to remove any endogenous ligand that might be complexed with the receptor in the cells. After 48 hours, either a saturation analysis to determine the K_d for tritiated dihydrotestosterone, [^{3}H]-DHT, or a competitive binding assay to evaluate the ability of test compounds to compete with [^{3}H]-DHT were performed. For the saturation analysis, media (RPMI 1640 or DMEM—0.2% CA-FBS) containing [^{3}H]-DHT (in concentrations ranging from 0.1 nM to 16 nM) in the absence (total binding) or presence (non-specific binding) of a 500-fold molar excess of unlabeled DHT were added to the cells. After 4 hours at 37° C., an aliquot of the total binding media at each concentration of [^{3}H]-DHT was removed to estimate the amount of free [^{3}H]-DHT. The remaining media was removed, cells were washed three times with PBS and harvested onto UniFilter GF/B plates (Packard), Microscint (Packard) was added and plates counted in a Top-Counter (Packard) to evaluate the amount of bound [^{3}H]-DHT.

For the saturation analysis, the difference between the total binding and the non-specific binding, was defined as specific binding. The specific binding was evaluated by Scatchard analysis to determine the K_d for [^{3}H]-DHT. See e.g. D. Rodbard, Mathematics and statistics of ligand assays: an illustrated guide: In: J. Langon and J. J. Clapp, eds., Ligand Assay, Masson Publishing U.S.A., Inc., New York, pp. 45–99, (1981), the disclosure of which is herein incorporated by reference.

For the competition studies, media containing 1 nM [^{3}H]-DHT and compounds of the invention ("test compounds") in concentrations ranging from 10^{-10} to 10^{-5} M were added to the cells. Two replicates were used for each sample. After 4 hours at 37° C., cells were washed, harvested and counted as described above. The data was plotted as the amount of [^{3}H]-DHT (% of control in the absence of test compound) remaining over the range of the dose response curve for a given compound. The concentration of test compound that inhibited 50% of the amount of [^{3}H]-DHT bound in the absence of competing ligand was quantified (IC_{50}) after log-logit transformation. The K_1 values were determined by application of the Cheng-Prusoff equation to the IC_{50} values, where:

$$K_1 = \frac{IC_{50}}{(1 + (^3\text{H-DHT})/K_d \text{ for } ^3\text{H-DHT})}.$$

After correcting for non-specific binding, IC_{50} values were determined. The IC_{50} is defined as the concentration of competing ligand needed to reduce specific binding by 50%. The K_ds for [^{3}H]-DHT for MDA 453 and LNCaP were 0.7 and 0.2 nM respectively.

Human Prostate Cell Proliferation Assay:

Compounds of the present invention were tested ("test compounds") on the proliferation of human prostate cancer cell lines. For that, MDA PCa2b cells, a cell line derived from the metastasis of a patient that failed castration, Navone et al., *Clin. Cancer Res.,* 3, 2493–500 (1997), were incubated with or without the test compounds for 72 hours and the amount of [^{3}H-thymidine incorporated into DNA was quantified as a way to assess number of cells and therefore proliferation. The MDA PCa2b cell line was maintained in BRFF-HPC 1 media (Biological Research Faculty & Facility Inc., MD) supplemented with 10% FBS. For the assay, cells were plated in Biocoated 96-well microplates and incubated at 37° C. in 10% FBS (charcoal-stripped)/BRFF-BMZERO (without androgens). After 24 hours, the cells were treated in the absence (blank) or presence of 1 nM DHT (control) or with test compounds (sample) of the present invention in concentrations ranging from 10^{-10} to 10^{-5} M. Duplicates were used for each sample. The compound dilutions were performed on a Biomek 2000 laboratory work station. Seventy two hours later 0.44 uCi. of [^{3}H]-Thymidine (Amersham) was added per well and incubated for another 24 h followed by tripsinization, harvesting of the cells onto GF/B filters. Micro-scint PS were added to the filters before counting them on a Beckman TopCount.

The % Inhibition was calculated as:

$$\% \text{ Inhibition}=100\times(1-[\text{average}_{control}-\text{average}_{blank}/\text{average}_{sample}-\text{average blank}])$$

Data was plotted and the concentration of compound that inhibited 50% of the [^{3}H]-Thymidine incorporation was quantified (IC_{50}).

C2C12 Mouse Myoblast Transactivation Assay:

Two functional transactivation assays were developed to assess the efficacy of androgen agonists in a muscle cell background using a luciferase reporter. The first assay (ARTA Stable 1) uses a cell line, Stable 1 (clone #72), which stably expresses the full length rat androgen receptor but requires the transient transfection of an enhancer/reporter. This cell line was derived from C2C12 mouse moyoblast cells. The second assay (ARTA Stable 2) uses a cell line, Stable 2 (clone #133), derived from Stable 1 which stably expresses both rAR and the enhancer/luciferase reporter.

The enhancer/reporter construct used in this system is pGL3/2XDR-1/luciferase. 2XDR-1 was reported to be an

AR specific response element in CV-1 cells, Brown et. al. *The Journal of Biological Chemisty* 272, 8227–8235, (1997). It was developed by random mutagenesis of an AR/GR consensus enhancer sequence.

ARTA Stable 1:

1. Stable 1 cells are plated in 96 well format at 6,000 cells/well in high glucose DMEM without phenol red (Gibco BRL, Cat. No.: 21063–029) containing 10% charcoal and dextran treated FBS (HyClone Cat. No.: SH30068.02), 50 mM HEPES Buffer (Gibco BRL, Cat. No.: 15630-080), 1× MEM Na Pyruvate (Gibco BRL, Cat. No.: 11360-070), 0.5× Antibiotic-Antimycotic, and 800 μg/ml Geneticin (Gibco BRL, Cat. No.: 10131-035).
2: 48 hours later, cells are transfected with pGL3/2XDR-1/luciferase using LipofectAMINE Plus™ Reagent (Gibco BRL, Cat. No.: 10964-013). Specifically, 5 ng/well pGL3/2XDR-1/luciferase DNA and 50 ng/well Salmon Sperm DNA (as carrier) are diluted with 5 μl/well Opti-MEMem media (Gibco BRL, Cat. No.: 31985-070). To this, 0.5 μl/well Plus reagent is added. This mixture is incubated for 15 minutes at room temperature. In a separate vessel, 0.385 μl/well LipofectAMINE reagent is diluted with 5 μl/well Opti-MEM. The DNA mixture is then combined with the LipofectAMINE mixture and incubated for an additional 15 minutes at room temperature. During this time, the media from the cells is removed and replaced with 60 μl/well of Opti-MEM. To this is added 10 μl/well of the DNA/LipofectAMINE transfection mixture. The cells are incubated for 4 hours.
3. The transfection mixture is removed from the cells and replaced with 90 μl of media as in #1 above.
4. 10 μl/well of appropriate drug dilution is placed in each well.
5. 24 hours later, the Steady-Glo™ Luciferase Assay System is used to detect activity according to the manufacturer's instructions (Promega, Cat. No.: E2520).

ARTA Stable 2

1. Stable 2 cells are plated in 96 well format at 6,000 cells/well in high glucose DMEM without phenol red (Gibco BRL, Cat. No.: 21063-029) containing 10% charcoal and dextran treated FBS (HyClone Cat. No.: SH30068.02), 50 mM HEPES Buffer (Gibco BRL, Cat. No.: 15630-080), 1×MEM Na Pyruvate (Gibco BRL, Cat. No.: 11360-070), 0.5× Antibiotic-Antimycotic, 800 μg/ml Geneticin (Gibco BRL, Cat. No.: 10131-035) and 800 μg/ml Hygromycin β (Gibco BRL, Cat. No.: 10687-010).
2. 48 hours later, the media on the cells is removed and replaced with 90 μl fresh. 10 μl/well of appropriate drug dilution is placed in each well.
3. 24 hours later, the Steady-Glo™ Luciferase Assay System is used to detect activity according to the manufacturer's instructions (Promega, Cat. No.: E2520).

See U.S. patent application Ser. No. 09/885,831, entitled "Cell Lines and Cell-BasedAssays for Identification of Androgen Receptor Modulators" filed Jun. 20, 2001 by Jacek Ostrowski et al. which Patent Application is incorporated herein by reference in its entirety.

Proliferation Assays

Murine Breast Cell Proliferation Assay:

The ability of compounds of the present invention ("test compounds") to modulate the function of the AR was determined by testing said compounds in a proliferation assay using the androgen responsive murine breast cell line derived from the Shionogi tumor, Hiraoka et al., *Cancer Res.,* 47, 6560–6564 (1987). Stable AR dependent clones of the parental Shionogi line were established by passing tumor fragments under the general procedures originally described in Tetuo, et. al., *Cancer Research* 25, 1168–1175 (1965). From the above procedure, one stable line, SC114, was isolated, characterized and utilized for the testing of example compounds. SC114 cells were incubated with or without the test compounds for 72 hours and the amount of [3H]-thymidine incorporated into DNA was quantified as a surrogate endpoint to assess the number of cells and therefore the proliferation rate as described in Suzuki et. al., *J. Steroid Biochem. Mol. Biol.* 37, 559–567 (1990). The SC114 cell line was maintained in MEM containing 10^{-8} M testosterone and 2% DCC-treated FCS. For the assay, cells were plated in 96-well microplates in the maintenance media and incubated at 37° C. On the following day, the medium was changed to serum free medium [Ham's F-12:MEM (1:1, v/v) containing 0.1% BSA] with (antagonist mode) or without (agonist mode) 10^{-8} M testosterone and the test compounds of the present invention in concentrations ranging from 10^{-10} to 10^{-5} M. Duplicates were used for each sample. The compound dilutions were performed on a Biomek 2000 laboratory work station. Seventy two hours later 0.44 uCi of [3H]-Thymidine (Amersham) was added per well and incubated for another 2 hr followed by tripsinization, and harvesting of the cells onto GF/B filters. Micro-scint PS were added to the filters before counting them on a Beckman TopCount.

For the antagonist mode, the % Inhibition was calculated as:

$$\%\ \text{Inhibition}=100\times(1-[\text{average}_{sample}-\text{average blank}/\text{average}_{control}-\text{average}_{blank}])$$

Data was plotted and the concentration of compound that inhibited 50% of the [^{3}H]-Thymidine incorporation was quantified (IC_{50}).

For the agonist mode % Control was referred as the effect of the tested compound compared to the maximal effect observed with the natural hormone, in this case DHT, and was calculated as:

$$\%\ \text{Control}=100\times(\text{average}_{sample}-\text{average}_{blank})/(\text{average}_{control}-\text{average}_{blank})$$

Data was plotted and the concentration of compound that inhibited 50% of the [^{3}H]-Thymidine incorporation was quantified (EC_{50}).

In Vitro Assay to Measure GR Induced AP-1 Transrepression:

The AP-1 assay is a cell based luciferase reporter assay. A549 cells, which contain endogenous glucocorticoid receptor, were stably transfected with an AP-1 DNA binding site attached to the luciferase gene. Cells are then grown in RPMI+10% fetal calf serum (charcoal-treated)+Penicillin/Streptomycin with 0.5 mg/ml geneticin. Cells are plated the day before the assay at approximately 40000 cells/well. On assay day, the media is removed by aspiration and 20 μl assay buffer (RPMI without phenol red+10% FCS (charcoal-treated)+Pen/Strep) is added to each well. At this point either 20 μl assay buffer (control experiments), the compounds of the present invention ("test compounds") (dissolved in DMSO and added at varying concentrations) or dexamethasome (100 nM in DMSO, positive control) are added to each well. The plates are then pre-incubated for 15 minutes at 37° C., followed by stimulation of the cells with 10 ng/ml PMA. The plates are then incubated for 7 hrs at 37° C. after which 40 μl luciferase substrate reagent is added to each well. Activity is measured by analysis in a luminometer as compared to control experiments treated with buffer or dexamethasome. Activity is designated as % inhibition of the reporter system as compared to the buffer control with 10

ng/ml PMA alone. The control, dexamethasone, at a concentration of $\leqq 10\ \mu M$ typically suppresses activity by 65%. Test compounds which demonstrate an inhibition of PMA induction of 50% or greater at a concentration of test compound of $\leqq 10\ \mu M$ are deemed active.

Wet Prostate Weight Assay AR Antagonist Assay:

The activity of compounds of the present invention as AR antagonists was investigated in an immature male rat model, a standard, recognized test of antiandrogen activity of a given compound, as described in L. G. Hershberger et al., *Proc. Soc. Expt. Biol. Med.,* 83, 175 (1953); P. C. Walsh and R. F. Gittes, "Inhibition of extratesticular stimuli to prostate growth in the castrated rat by antiandrogens", *Endocrinology,* 86, 624 (1970); and B. J. Furr et al., "ICI 176,334: A novel non-steroid, peripherally selective antiandrogen", *J. Endocrinol.,* 113, R7–9 (1987), the disclosures of which are herein incorporated by reference.

The basis of this assay is the fact that male sexual accessory organs, such as the prostate and seminal vesicles, play an important role in reproductive function. These glands are stimulated to grow and are maintained in size and secretory function by the continued presence of serum testosterone (T), which is the major serum androgen (>95%) produced by the Leydig cells in the testis under the control of the pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH). Testosterone is converted to the more active form, dihydrotestosterone, (DHT), within the prostate by 5α-reductase. Adrenal androgens also contribute about 20% of total DHT in the rat prostate, compared to 40% of that in 65-year-old men. F. Labrie et al. Clin. Invest. Med., 16, 475–492 (1993). However, this is not a major pathway, since in both animals and humans, castration leads to almost complete involution of the prostate and seminal vesicles without concomitant adrenalectomy. Therefore, under normal conditions, the adrenals do not support significant growth of prostate tissues. M. C. Luke and D. S. Coffey, "*The Physiology of Reproduction*" ed. By E. Knobil and J. D. Neill, 1, 1435–1487 (1994). Since the male sex organs are the tissues most responsive to modulation of the androgen activity, this model is used to determine the androgen dependent growth of the sex accessory organs in immature castrated rats.

Male immature rats (19–20 days old Sprague-Dawley, Harlan Sprague-Dawely) were castrated under metofane ansestesia. Five days after surgery these castrated rats (60–70 g, 23–25 day-old) were dosed for 3 days. Animals were dosed sub-cutaneously (s.c.) 1 mg/kg with Testosterone Proprionate (TP) in arachis oil vehicle and antiandrogen test compounds (compounds of the present invention) were dosed orally by gavage (p.o.) in dissolved/suspensions of 80% PEG 400 and 20% Tween 80 (PEGTW). Animals were dosed (v/w) at 0.5 ml of vehicle/100 g body weight. Experimental groups were as follows:

1. Control vehicle
2. Testosterone Propionate (TP) (3 mg/rat/day, subcutaneous)
3. TP plus Casodex (administered p.o. in PEGTW, QD), a recognized antiandrogen, as a reference compound.
4. To demonstrate antagonist activity, a compound of the present invention ("test compound") was administered (p.o. in PEGTW, QD) with TP (s.c. as administered in group 2) in a range of doses.
5. To demonstrate agonist activity a compound of the present invention ("test compound") was administered alone (p.o. in PEGTW, QD) in a range of doses.

At the end of the 3-day treatment, the animals were sacrificed, and the ventral prostate weighed. To compare data from different experiments, the sexual organs weights were first standardized as mg per 100 g of body weight, and the increase in organ weight induced by TP was considered as the maximum increase (100%). ANOVA followed by one-tailed Student or Fischer's exact test was used for statistical analysis.

The gain and loss of sexual organ weight reflect the changes of the cell number (DNA content) and cell mass (protein content), depending upon the serum androgen concentration. See Y. Okuda et al., *J. Urol.,* 145, 188–191 (1991), the disclosure of which is herein incorporated by reference. Therefore, measurement of organ wet weight is sufficient to indicate the bioactivity of androgens and androgen antagonist. In immature castrated rats, replacement of exogenous androgens increases seminal vesicles (SV) and the ventral prostate (VP) in a dose dependent manner.

The maximum increase in organ weight was 4 to 5-fold when dosing 3 mg/rat/day of testosterone (T) or 1 mg/rat/day of testosterone propionate (TP) for 3 days. The EC_{50} of T and TP were about 1 mg and 0.03 mg, respectively. The increase in the weight of the VP and SV also correlated with the increase in the serum T and DHT concentration. Although administration of T showed 5-times higher serum concentrations of T and DHT at 2 hours after subcutaneous injection than that of TP, thereafter, these high levels declined very rapidly. In contrast, the serum concentrations of T and DHT in TP-treated animals were fairly consistent during the 24 hours, and therefore, TP showed about 10–30-fold higher potency than free T.

In this immature castrated rat model, a known AR antagonist (Casodex) was also administered simultaneously with 0.1 mg of TP (ED_{80}), inhibiting the testosterone-mediated increase in the weights of the VP and SV in a dose dependent manner. The antagonist effects-were similar when dosing orally or subcutaneously. Compounds of the invention also exhibited AR antagonist activity by suppressing the testosterone-mediated increase in the weights of VP and SV.

Levator Ani & Wet Prostate Weight Assay AR Agonist Assay:

The activity of compounds of the present invention as AR agonists was investigated in an immature male rat model, a recognized test of anabolic effects in muscle and sustaining effects in sex organs for a given compound, as described in L. G. Hershberger et al., *Proc. Soc. Expt. Biol. Med.,* 83, 175 (1953); B. L. Beyler et al, "Methods for evaluating anabolic and catabolic agents in laboratory animals", *J. Amer. Med. Women's Ass.,* 23, 708 (1968); H. Fukuda et al., "Investigations of the levator ani muscle as an anabolic steroid assay", *Nago Dai. Yak. Ken. Nem.* 14, 84 (1966) the disclosures of which are herein incorporated by reference.

The basis of this assay lies in the well-defined action of androgenic agents on the maintenance and growth of muscle tissues and sexual accessory organs in animals and man. Androgenic steroids, such as testosterone (T), have been well characterized for their ability to maintain muscle mass. Treatment of animals or humans after castrations with an exogenous source of T results in a reversal of muscular atrophy. The effects of T on muscular atrophy in the rat levator ani muscle have been well characterized. M. Masuoka et al., "Constant cell population in normal, testosterone deprived and testosterone stimulated levator ani muscles" *Am. J. Anat.* 119, 263 (1966); Z. Gori et al., "Testosterone hypertrophy of levator ani muscle of castrated rats. I. Quantitative data" *Boll.-Soc. Ital. Biol. Sper.* 42, 1596 (1966); Z. Gori et al., "Testosterone hypertrophy of levator ani muscle of castrated rats. II. Electron-microscopic observations" *Boll.-Soc. Ital. Biol. Sper.* 42, 1600 (1966); A. Boris

et al., *Steroids* 15, 61 (1970). As described above, the effects of androgens on maintenance of male sexual accessory organs, such as the prostate and seminal vesicles, is well described. Castration results in rapid involution and atrophy of the prostate and seminal vesicles. This effect can be reversed by exogenous addition of androgens. Since both the levator ani muscle and the male sex organs are the tissues most responsive to the effects of androgenic agents, this model is used to determine the androgen dependent reversal of atrophy in the levator ani muscle and the sex accessory organs in immature castrated rats. Sexually mature rats (200–250 g, 6–8 weeks-old, Sprague-Dawley, Harlan) were acquired castrated from the vendor (Taconic). The rats were divided into groups and treated daily for 7 to 14 days with one of the following:

1. Control vehicle
2. Testosterone Propionate (TP) (3 mg/rat/day, subcutaneous)
3. TP plus Casodex (administered p.o. in PEGTW, QD), a recognized antiandrogen, as a reference compound.
4. To demonstrate antagonist activity, a compound of the present invention ("test compound") was administered (p.o. in PEGTW, QD) with TP (s.c. as administered in group 2) in a range of doses.
5. To demonstrate agonist activity a compound of the present invention ("test compound") was administered alone (p.o. in PEGTW, QD) in a range of doses.

At the end of the 7–14-day treatment, the animals were sacrificed by carbon dioxide, and the levator ani, seminal vesicle and ventral prostate weighed. To compare data from different experiments, the levator ani muscle and sexual organ weights were first standardized as mg per 100 g of body weight, and the increase in organ weight induced by TP was considered as the maximum increase (100%). Super-anova (one factor) was used for statistical analysis.

The gain and loss of sexual organ weight reflect the changes of the cell number (DNA content) and cell mass (protein content), depending upon the serum androgen concentration. See Y. Okuda et al., *J. Urol.,* 145, 188–191 (1991), the disclosure of which is herein incorporated by reference. Therefore, measurement of organ wet weight is sufficient to indicate the bioactivity of androgens and androgen antagonist. In immature castrated rats, replacement of exogenous androgens increases levator ani, seminal vesicles (SV) and prostate in a dose dependent manner.

The maximum increase in organ weight was 4 to 5-fold when dosing 3 mg/rat/day of testosterone (T) or 1 mg/rat/day of testosterone propionate (TP) for 3 days. The EC_{50} of T and TP were about 1 mg and 0.03 mg, respectively. The increase in the weight of the VP and SV also correlated with the increase in the serum T and DHT concentration. Although administration of T showed 5-times higher serum concentrations of T and DHT at 2 hours after subcutaneous injection than that of TP, thereafter, these high levels declined very rapidly. In contrast, the serum concentrations of T and DHT in TP-treated animals were fairly consistent during the 24 hours, and therefore, TP showed about 10–30-fold higher potency than free T.

MDA PCa2b Human Prostate Zenograft Assay:

In Vivo Antitumor Testing: MDA-PCa-2b human prostate tumors were maintained in Balb/c nu/nu nude mice. Tumors were propagated as subcutaneous transplants in adult male nude mice (4–6 weeks old) using tumor fragments obtained from donor mice. Tumor passage occurred every 5–6 weeks.

For antitumor efficacy trial, the required number of animals needed to detect a meaningful response were pooled at the start of the experiment and each was given a subcutaneous implant of a tumor fragment (~50 mg) with a 13-gauge trocar. Tumors were allowed to grow to approx. 100–200 mg (tumors outside the range were excluded) and animals were evenly distributed to various treatment and control groups. Treatment of each animal was based on individual body weight. Treated animals were checked daily for treatment related toxicity/mortality. Each group of animals was weighed before the initiation of treatment (Wt1) and then again following the last treatment dose (Wt2). The difference in body weight (Wt2−Wt1) provides a measure of treatment-related toxicity.

Tumor response was determined by measurement of tumors with a caliper twice a week, until the tumors reach a predetermined "target" size of 0.5 gm. Tumor weights (mg) were estimated from the formula: Tumor weight=(length×width2)÷2.

Tumor response end-point was expressed in terms of tumor growth inhibition (% T/C), defined as the ratio of median tumor weights of the treated tumors (T) to that of the control group (C).

To estimate tumor cell kill, the tumor volume doubling time was first calculated with the formula:

TVDT=Median time (days) for control tumors to reach target size−Median time (days) for control tumors to reach half the target size *s* And, Log cell kill=(*T*−*C*)÷(3.32×*TVDT*)

Statistical evaluations of data were performed using Gehan's generalized Wilcoxon test.

Dunning Prostate Tumor:

Dunning R3327H prostate tumor is a spontaneously derived, well differentiated androgen responsive adenocarcinoma of the prostate (Smolev J K, Heston W D, Scott W W, and Coffey D S, *Cancer Treat Rep.* 61, 273–287 (1977)). The growth of the R3327H subline has been selected for its highly androgen-dependent and reproducible growth in intact male rats. Therefore, this model and other sublines of this tumor have been widely used to evaluate in vivo antitumor activities of antiandrogens such as flutamide and bacilutamide/Casodex (Maucher A., and von Angerer, *J. Cancer Res. Clin. Oncol.,* 119, 669–674 (1993), Furr B. J. A. *Euro. URL.* 18 (suppl. 3), 2–9 (1990), Shain S. A. and Huot R I. *J. Steriod Biochem.* 31, 711–718 (1988)).

At the beginning of the study, the Dunning tumor pieces (about 4×4 mm) are transplanted subcutaneously to the flank of mature male Copenhagen rats (6–7 weeks old, Harlan-Sprague Dawley, Indianapolis, Md.). About 6 weeks after the implantation, the animals with tumors of measurable size (about 80–120 mm^2) are randomized into treatment groups (8–10 rats/group) and the treatments are initiated. One group of the rats are castrated to serve as the negative control of tumor growth. Animals are treated daily with compounds of the current invention, standard antiandrogens such as bacilutamide or vehicle (control) for an average of 10 to 14 weeks. Test compounds are dissolved in a vehicle of (2.5 ml/kg of body weight) 10% polyethylene glycol and 0.05% Tween-80 in 1% carboxymethyl cellulose, PEG/CMC, (Sigma, St Louis, Mo.). Typical therapeutic experiments would include three groups of three escalating doses for each standard or test compound (in a range of 300–3 mg/kg).

Tumors in the vehicle (control) group reach a size of 1500 to 2500 mm^3, whereas the castrated animal group typically shows tumor stasis over the 14 weeks of observation. Animals treated orally with 20 mg/kg of bicalutamide or flutamide would be expected to show a 40% reduction in tumor volumes compared to control after 14 weeks of treatment. The size of tumors are measured weekly by

vernier caliper (Froboz, Switzerland), taking perpendicular measurements of length and width. Tumor volumes are measured in mm^3 using the formula: Length×Width×Height=Volume. Statistical differences between treatment groups and control are evaluated using multiple ANOVA analysis followed by one tail non-parametric Student t test.

Mature Rat Prostate Weight Assay:

The activity of compounds of the present invention were investigated in a mature male rat model, which is a variation of the Levator ani & wet prostate weight assay described above. The above in vivo assays are recognized assays for determining the anabolic effects in muscle and sustaining effects in sex organs for a given compound, as described in L. G. Hershberger et al., 83 *Proc. Soc. Expt. Biol. Med.,* 175 (1953); B. L. Beyler et al, "Methods for evaluating anabolic and catabolic agents in laboratory animals", 23 *J. Amer. Med. Women's Ass.,* 708 (1968); H. Fukuda et al., "Investigations of the levator ani muscle as an anabolic steroid assay", 14 *Nago Dai. Yak. Ken. Nem.* 84 (1966) the disclosures of which are herein incorporated by reference. The basis of this assay lies in the well-defined action of androgenic agents on the maintenance and growth of muscle tissues and sexual accessory organs in animals and man.

The male sexual accessory organs, such as the prostate and seminal vesicles, play an important role in reproductive function. These glands are stimulated to grow and are maintained in size and secretory function by the continued presence of serum testosterone (T), which is the major serum androgen (>95%) produced by the Leydig cells in the testis under the control of the pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH). Testosterone is converted to the more active form, dihydrotestosterone, (DHT), within the prostate by 5α-reductase. Adrenal androgens also contribute about 20% of total DHT in the rat prostate, compared to 40% of that in 65-year-old men. F. Labrie et. al. 16 *Clin. Invest. Med.,* 475–492 (1993). However, this is not a major pathway, since in both animals and humans, castration leads to almost complete involution of the prostate and seminal vesicles without concomitant adrenalectomy. Therefore, under normal conditions, the adrenals do not support significant growth of prostate tissues, M. C. Luke and D. S. Coffey, "The Physiology of Reproduction" ed. By E. Knobil and J. D. Neill, 1, 1435–1487 (1994). Since the male sex organs and the levator ani are the tissues most responsive to modulation of the androgen activity, this model is used to determine the activity of compounds that modulate the androgen receptor pathway in mature rats.

Along with its mitogenic activity on tissues such as prostate, seminal vesicle and muscle, testosterone also serves as a negative regulator for its own biosynthesis. Testosterone production in the Leydig cells of the testis is controlled by the level of circulating LH released from the pituitary gland. LH levels are themselves controlled by the level of LHRH produced in the hypothalmic region. Testosterone levels in the blood serve to inhibit the secretion of LHRH and subsequently reduce levels of LH and ultimately the levels of circulating testosterone levels. By measuring blood levels of LH as they are effected by compounds of the present invention ("test compounds"), it is possible to determine the level of agonist or antagonist activity of said compounds at the hypothalamic axis of this endocrine cycle.

Matched sets of Harlan Sprague-Dawely rats (40–42 days old, 180–220 g), were dosed orally by gavage (p.o.) with the test compounds in dissolved/suspensions of 80% PEG 400 and 20% Tween 20 (PEGTW) for 14 days. Two control groups, one intact and one castrated were dose orally only with the PEGTW vehicle. Animals were dosed (v/w) at 0.5 ml of vehicle/100 g body weight. Experimental groups were as follows:

1. Intact vehicle (p.o., PEGTW, QD)
2. Control vehicle (p.o., PEGTW, QD)
3. Bicalutamide (Casodex, a recognized antiandrogen, as a reference compound) or a compound of the present invention, p.o. in PEGTW QD. (in a range of doses). At the end of the 14-day treatment, the animals were sacrificed, and the ventral prostate, the seminal vesicles, and the levator ani were removed surgically and weighed. To compare data from different experiments, the organs weights were first standardized as mg per 100 g of body weight, and expressed as a percentage of the value of the respective organ in the intact group.

Rat luteinizing hormone (rLH) is quantitatively determined with the Biotrak [125I] kit (Amersham Pharmacia Biotek), following the manufacturer directions. The assay is based on the competition by the LH present in the serum of the binding of [^{125}I] rLH to an Amerlex-M bead/antibody suspension. The radioactivity that remains after incubation with the serum and subsequent washes is extrapolated into a standard curve to obtain a reading in ng/ml.

The gain and loss of sexual organ and levator ani weight reflect the changes of the cell number (DNA content) and cell mass (protein content), depending upon the serum androgen concentration, see Y. Okuda et al., *J. Urol.,* 145, 188–191 (1991), the disclosure of which in herein incorporated by reference. Therefore, measurement of organ wet weight is sufficient to indicate the bioactivity of androgens and androgen antagonist. In the mature rats assay, active agonist agents will have no effect or will increase the weight of one or more of the androgen responsive organs (levator ani, prostate, seminal vessicle) and will have no effect or a suppressive effect on LH secretion. Compounds with antagonist activity will decrease the weight of one or more of the androgen responsive organs (levator ani, prostate, seminal vesicle) and will have no effect or a reduced suppressive effect on LH secretion.

CWR22 Human Prostate Zenograft Assay:

In Vivo Antitumor Testing: CWR22 human prostate tumors were maintained in Balb/c nu/nu nude mice. Tumors were propagated as subcutaneous transplants in adult male nude mice (4–6 weeks old) using tumor fragments obtained from donor mice. Tumor passage occurred every 5–6 weeks.

For antitumor efficacy trial, the required number of animals needed to detect a meaningful response were pooled at the start of the experiment and each was given a subcutaneous implant of a tumor fragment (~50 mg) with a 13-gauge trocar. Tumors were allowed to grow to approx. 100–200 mg (tumors outside the range were excluded) and animals were evenly distributed to various treatment and control groups. Treatment of each animal was based on individual body weight. Treated animals were checked daily for treatment related toxicity/mortality. Each group of animals was weighed before the initiation of treatment (Wt1) and then again following the last treatment dose (Wt2). The difference in body weight (Wt2−Wt1) provides a measure of treatment-related toxicity.

Tumor response was determined by measurement of tumors with a caliper twice a week, until the tumors reach a predetermined "target" size of 0.5 gm. Tumor weights (mg) were estimated from the formula: Tumor weight=(length×width2)÷2.

Tumor response end-point was expressed in terms of tumor growth inhibition (% T/C), defined as the ratio of median tumor weights of the treated tumors (T) to that of the control group (C).

To estimate tumor cell kill, the tumor volume doubling time was first calculated with the formula:

TVDT=Median time (days) for control tumors to reach target size–Median time (days) for control tumors to reach half the target size And, Log cell kill=$(T-C)\div(3.32\times TVDT)$

Statistical evaluations of data were performed using Gehan's generalized Wilcoxon test.

The following Examples illustrate embodiments of the present invention, and are not intended to limit the scope of the claims. Within certain Examples, one compound of the formula I is prepared and then employed to further prepare one or more additional compounds of the formula I or salts thereof. Methods employed to prepare one compound of the formula I or salt thereof as described herein can be employed as appropriate to prepare other compounds of the invention.

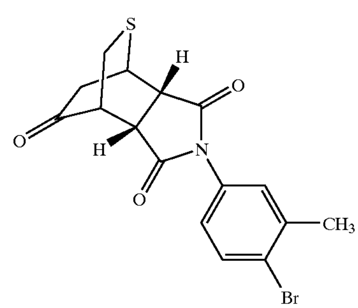
ABBREVIATIONS

The following abbreviations are used herein:

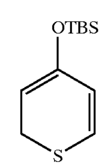
DBU=1,8-diazabicyclo[5.4.0]undec-7-ene
4-DMAP=4-dimethylaminopyridine
ee=enantiomeric excess
DMF dimethylformamide
EtOAc=ethyl acetate
LDA=lithium diisopropylamide
Hünig's Base=N,N-diisopropylethylamine
Me=methyl
RT=retention time
TFA=trifluoroacetic acid
THF=tetrahydrofuran
TLC=thin layer chromatography
TMS=trimethylsilyl
pTSA=para-toluenesulfonic acid
⊗=heat
t-Bu=tert-butyl
$PhCH_3$=toluene
Pd/C palladium on activated charcoal
TsCl=tosyl chloride
TBSOTf=tert-butyldimethylsilyl trifluoromethane sulfonate
TBS=tert-butyldimethylsilane
MeI methyl iodide
$(BOC)_2O$=di-tert-butyl dicarbonate
TEA=triethylamine
n-BuLi=n-butyllithium
rt=room temperature
LC=liquid chromatography
Ts=tosyl
Ph=phenyl
EtOH=ethanol
DCE=dichloroethane
DMSO=dimethylsulfoxide
Ra-Ni=Raney Nickel
MS=molecular sieves
MS(ES)=Electro-Spray Mass Spectrometry
mCPBA=m-chloroperoxybenzoic acid
sat=saturated
AcOH=acetic acid
MeOH=methanol
Et_2O=diethyl ether
Ac=acetyl
DEAD=diethyl azodicarboxylate
h=hours
Et=ethyl
WSDCC=water soluble dicarbonyl diimide, 1-(3-di methylaminopropyl)-3-ethylcarbodiimide hydrochloride
TBAF=tetrabutylammonium fluoride
DBAD=di-terbutylazodicarboxylate
DCC=Dicyclohexylcarbodiimide
Wilkinson's catalyst=RhCl(PPh3)3
ADDP=1,1-[azodicarbonyl]dipiperidine
DMA=dimethylacetamide
DME=1,2-dimethoxyethane
BOP=benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate
HRMS=high resolution mass spectrometry
TBME=MTBE=methyl tert-butyl ether (i.e., 2-methoxy-2-methyl-propane)
$TiCl_2\ Cp_2$=bis(cyclopentadienyl)titanium dichloride
DPPA=diphenylphosphoryl azide
HMPA=hexamethylphosphoryl amide
V %=volume percent
BH_3.DMS=borane dimethylsulfate
vvm=volume gas per volume liquid per minute

EXAMPLE 1

(3aα,4α,7α,7aα)-2-(4-Bromo-3-methylphenyl) tetrahydro-4,7-ethanothiopyrano[3,4-c]pyrrole-1,3,8 (2H,4H)-trione (1C)



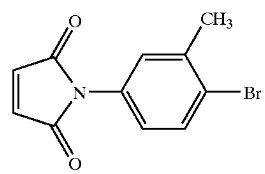
A. 4-(tert-Butyldimethylsiloxy)-2H-thiopyran (1A)



2,3-Dihydro-4H-thiopyran-4-one (1.50 g, 13.1 mmol, synthesized as described in Richards et al. *J. Org. Chem.* 46, 4836–4842 (1981)) was dissolved in CH_2Cl_2 (130 mL) and

triethylamine (5.47 mL, 39.4 mmol) was added. tert-Butyldimethylsilyl trifluoromethanesulfonate (3.62 mL, 15.8 mmol) was then added. After 10 minutes, the volatiles were removed in vacuo at 25° C. The resulting yellow oil was passed through a short column of SiO_2 eluting with 3% TEA in hexanes to yield 1.82 g (7.97 mmol, 61%) of compound 1A as an orange oil.

B. 1-[4-bromo-3-methylphenyl]-1H-pyrrole-2,5-dione (1B)



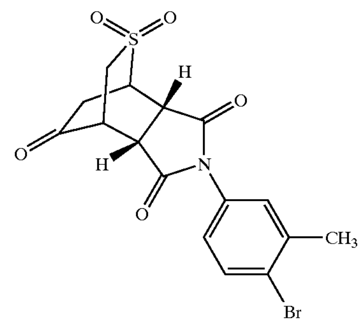
4-Bromo-3-methylaniline (1.55 g, 8.33 mmol) and maleic anhydride (0.898 g, 9.16 mmol) were dissolved in acetic acid (10 mL) and heated at 115° C. for 12 h. The reaction was then cooled to 25° C. and the acetic acid was removed in vacuo. The resulting residue was suspended in 5% K_2CO_3 (100 mL), stirred for 25 minutes, filtered and rinsed with water. The material was then dried in vacuo to give 1.65 g (6.20 mmol, 74%) of compound 1B as a light brown solid. HPLC: 100% at 2.96 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm).

C. (3aα,4α,7α,7aα)-2-(4-Bromo-3-methylphenyl) tetrahydro-4,7-ethanothiopyrano[3,4-c]pyrrole-1,3,8 (2H,4H)-trione (1C)

Compound 1A (0.313 g, 1.41 mmol) and compound 1B (0.250 g, 0.940 mmol) were dissolved in toluene and heated to reflux for 5 h. The toluene was then removed by passing a stream of argon through the reaction flask. The residue was then purified by flash chromatography on SiO_2 eluting with 20% hexane in chloroform. This gave 0.168 g of the enol ether intermediate as a yellow solid. The enol ether intermediate was dissolved in dichloroethane (2.0 mL) and TFA (0.25 mL) was added. After 0.5 h, the reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (2×30 mL). The organics were dried over anhydrous sodium sulfate and evaporated to give 0.079 g (0.21 mmol, 22%) of compound 1C as a white solid. HPLC: 99% at 3.010 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 396.9 $[M+NH_4]^+$.

EXAMPLE 2

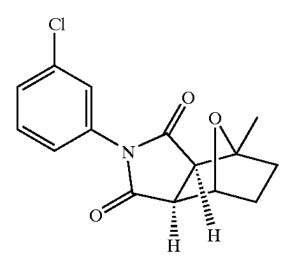
(3aα,4α,7α,7aα)-2-(4-Bromo-3-methylphenyl) tetrahydro-4,7-ethanothiopyrano[3,4-c]pyrrole-1,3,8 (2H,4H)-trione 5,5-dioxide (2)



Compound 1C (0.040 g, 0.11 mmol) was dissolved in CH_2Cl_2 (4.0 mL) and cooled to 0° C. m-CPBA (60% purity, 0.061 g, 0.21 mmol) was added and the reaction was then warmed to 25° C. After 1 h, a 1:1 mixture of saturated $NaHCO_3$ and saturated sodium sulfite (20 mL) was added with vigorous stirring. After 15 minutes, the mixture was extracted with CH_2Cl_2 (2×30 mL) and the organics were dried over anhydrous sodium sulfate to yield 0.031 g (0.075 mmol, 71%) of compound 2 as a white solid. No purification was necessary. HPLC: 78% at 2.290 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 429.8 $[M+NH_4]^+$.

EXAMPLE 3

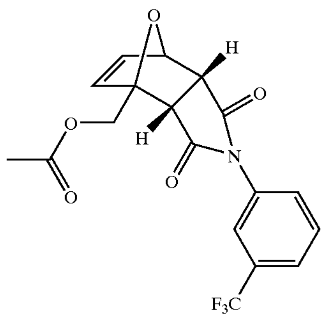
(3aα,4β,7β,7aα)-2-(3-Chlorophenyl)hexahydro-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (3)

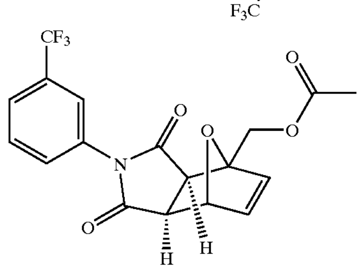


3-Chloroaniline (0.100 g, 0.787 mmol) and 3,6-endoxo-3-methylhexahydrophthalic anhydride (0.172 g, 0.945 mmol) were dissolved in AcOH (2.0 mL) and heated at 110° C. for 11 h. The reaction was then cooled to 25° C., poured into cold saturated aq. K_2CO_3 and stirred vigorously for 10 min. The solution was then filtered and rinsed with water. The resulting filtrate was dried in vacuo to give 0.118 g (0.404 mmol, 51%) of compound 3 as a white solid. No further purification was needed. HPLC: 99% at 2.510 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 292.32 $[M+H]^+$.

EXAMPLE 4

(3aα,4α,7α,7aα)- and (3aα,4β,7β,7aα)-4-[(Acetyloxy)methyl]-3a,4,7,7a-tetrahydro-2-[3-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione (4i and 4ii, Respectively)

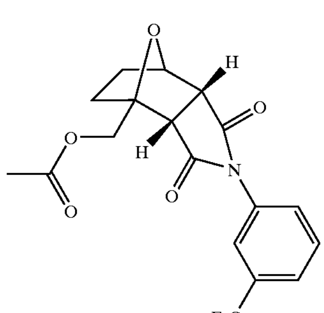




2-Acetoxymethylfuran (0.599 mL, 4.78 mmol) and 1-[3-(trifluoromethyl)-phenyl]-1H-pyrrole-2,5-dione (0.500 g, 2.39 mmol, prepared as described in Example B) were dissolved in methylene chloride (3.0 mL) at 25° C. After 22 h, the volatiles were removed in vacuo and the resulting residue was purified by flash chromatography on SiO_2 eluting with 0–15% acetone in methylene chloride to give 0.438 g (1.15 mmol, 48%) of a yellow oil as a 2:1 mixture of compound 4i and compound 4ii, which was not separated. HPLC: 100% at 3.093 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 398.9 $[M+NH_4]^+$.

EXAMPLE 5

(3aα,4α,7α,7aα)- and (3aα,4β,7β,7aα)-4-[(Acetyloxy)methyl]-Hexahydro-2-[3-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione (5i and 5ii, Respectively)



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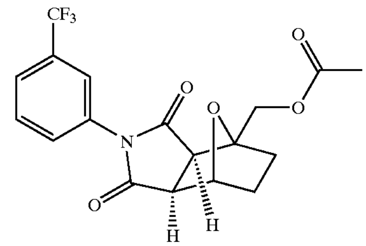
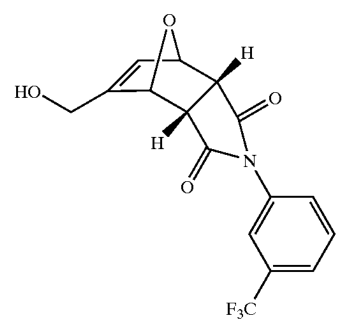
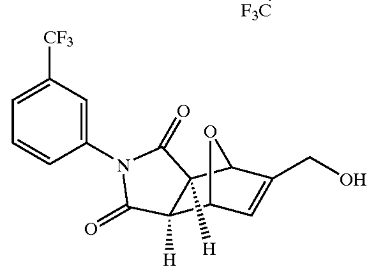


The 2:1 mixture of compounds 4i and 4ii (0.361 g, 0.948 mmol) was dissolved in ethyl acetate (25 mL) and Pd/C (10% Pd, 0.2 g) was added. Hydrogen was introduced via a balloon and the reaction was stirred at 25° C. for 4 h, followed by filtration through Celite and rinsing with ethyl acetate. Concentration in vacuo gave 0.348 g (0.908 mmol, 96%) of a yellow oil that was determined to be a 2:1 mixture of compound 5i and compound 5ii (which was not separated). HPLC: 100% at 2.900 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 401.0 $[M+NH_4]^+$.

EXAMPLE 6

(3aα,4α,7α,7aα)- and (3aα,4β,7β,7aα)-3a,4,7,7a-Tetrahydro-5-(hydroxymethyl)-2-[3-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione (6i and 6ii, Respectively)



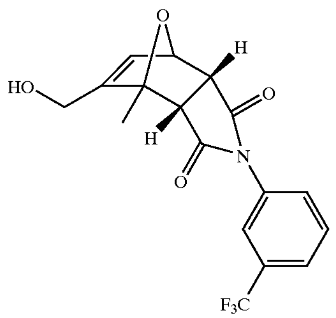


1-[3-(Trifluoromethyl)phenyl]-1H-pyrrole-2,5-dione (0.500 g, 2.39 mmol, prepared as described in Example 1B) and 3-furanmethanol (0.412 mL, 4.78 mmol) were dissolved in methylene chloride (3.0 mL) and stirred at 25° C. for 20 h. The volatiles were then removed in vacuo and the resulting material purified by flash chromatography on SiO_2 eluting with chloroform/acetone to give 0.379 g (1.12 mmol, 47%) of compound 6i and 0.220 g of compound 6ii, both as white solids. Compound 6i: HPLC: 100% at 2.197 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing

0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 338.0 $[M-H]^-$. Compound 6ii: HPLC: 100% at 2.477 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 338.0 $[M-H]^-$.

EXAMPLE 7

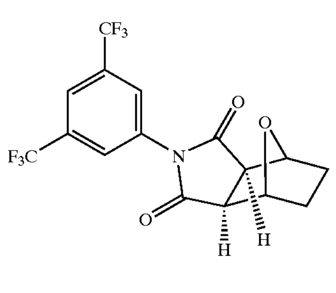
(3aα,4α,7α,7aα)-3a,4,7,7a-Tetrahydro-5-(hydroxymethyl)-4-methyl-2-[3-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione (7)



2-Methyl-3-furanmethanol (0.537 g, 4.78 mmol) and 1-[3-(trifluoromethyl)-phenyl]-1H-pyrrole-2,5-dione (0.500 g, 2.39 mmol, prepared as described in Example 1B) were dissolved in dichloroethane (2.0 mL) and stirred at 25° C. for 20 h. The reaction was then concentrated in vacuo and purified by flash chromatography in SiO_2 eluting with ethyl acetate/methylene chloride to give 0.317 g (0.897 mmol, 37.5%) of compound 7 as a white solid. No other possible isomer was isolated after chromatography. HPLC: 100% at 2.197 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 351.9 $[M-H]^-$.

EXAMPLE 8

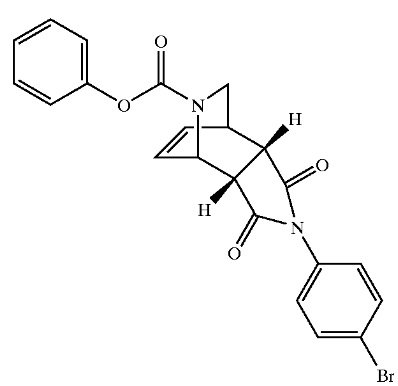
(3aα,4β,7β,7aα)-2-[3,5-Bis(trifluoromethyl)phenyl]hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione (8)



3,5-Bis(trifluoromethyl)aniline (0.017 g, 0.075 mmol) was dissolved in acetic acid (0.300 mL) and transferred to a 1.5 mL conical vial with a septa cap. Stock solutions of an additional 95 amines were prepared as described above. To each of the above vials was added 0.40 mL (0.12 mmol) of a stock solution of exo-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride in acetic acid. The vials were then sealed and heated at 110° C. for 11 h. Upon cooling to 25° C., the caps were removed from the vials and the acetic acid was removed in vacuo. To each vial was added 1 mL of 2:1 acetone/methylene chloride and the vials were heated at 40° C. for 1 h. Once all products were in solution, they were transferred via robot to filter tubes with coarse frits pre-wetted with 0.2 mL of water. Nitrogen was blown through each tube until the volatile organics were removed. 1.5 mL of 10% aq. K_2CO_3 was then added to each tube followed by vigorous shaking at 25° C. for 15 min. The tubes were then drained, resealed and 1.0 mL of water was added to each tube followed by shaking. The tubes were drained again and washed with water a second time. The resulting residues in each tube were then dried in vacuo for 48 h. After drying, 1.0 mL of 20% TFA in methylene chloride was added to each tube and the racks were shaken for 30 min. The tubes were then drained into a 96-well plate with pre-tared custom micro-tubes present. Each tube was assayed for product purity (analytical LC) and identity (LC-MS). The tubes were then concentrated in vacuo and weighed for yields. The tube containing the reaction of 3,5-bistrifluoromethylaniline and exo-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride, yielded 0.022 g (0.058 mmol, 77%) of compound 8 as a white solid. HPLC: 94% at 4.03 nin (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 434.2 $[M+Na+MeOH]^+$. Of the remaining 95 additional reactions run, a total of 80 final compounds were obtained in >70% purity and >5 mg yield. Several samples needed further purification which was performed by short SiO_2 column eluting with methylene chloride/acetone. See Table 2 below.

EXAMPLE 9

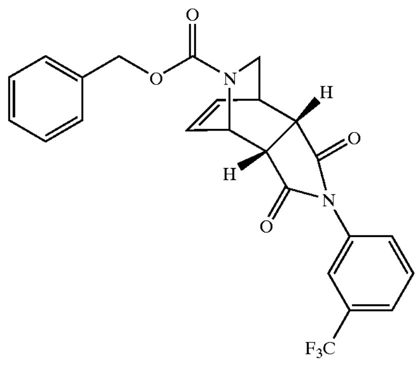
(3aα,4α,7α,7aα)-2-(4-Bromophenyl)octahydro-1,3-dioxo-4,7-etheno-5H-pyrrolo[3,4-c]pyridine-5-carboxylic Acid Phenyl Ester (9)



1-[4-Bromophenyl]-1H-pyrrole-2,5-dione (0.250 g, 0.992 mmol, prepared as described in Example 1B) and 1(2H)-pyridinecarboxylic acid phenylmethyl ester (0.299 g, 1.49 mmol, synthesized as described in Richard et al. *J. Org. Chem.* 46, 4836–4842 (1981)) were dissolved in toluene and heated at 85° C. for 1 h. Upon cooling to 25° C., the toluene was removed in vacuo. The resulting residue was dissolved in a minimum amount of chloroform and the product was precipitated by addition of hexanes. After 1 h at 25° C., the product was filtered and rinsed with cold 20% hexanes in chloroform giving 0.243 g (0.536 mmol, 54%) of compound 9 as a white solid (single isomer). HPLC: 100% at 3.393 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 454.98 $[M+H]^+$.

EXAMPLE 10

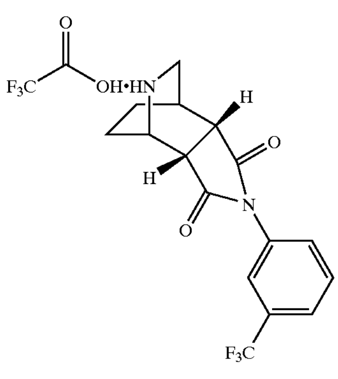
(3aα,4α,7α,7aα)-2-(4-Bromophenyl)octahydro-1,3-dioxo-4,7-etheno-5H-pyrrolo[3,4-c]pyridine-5-carboxylic Acid Phenylmethyl Ester (10)



1-[3-(Trifluoromethyl)phenyl]-1H-pyrrole-2,5-dione (3.78 g, 15.7 mmol, prepared as described in Example 1B) and 1(2H)-pyridinecarboxylic acid phenylmethyl ester (4.00 g, 18.8 mmol, synthesized as described in Richard et al., *J. Org. Chem.* 46, 4836–4842 (1981)) were dissolved in toluene and heated at 80° C. for 3 h. After cooling to 25° C., the toluene was removed in vacuo and the resulting residue was purified by flash chromatography on SiO_2 eluting with methanol/methylene chloride to give 3.20 g (7.01 mmol, 45%) of compound 10 as a yellow oil. HPLC: 95% at 3.510 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 457.2 $[M+H]^+$.

EXAMPLE 11

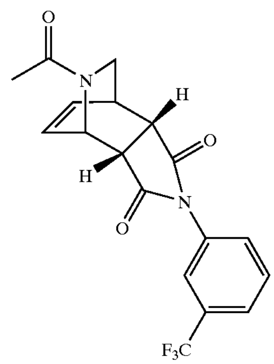
(3aα,4α,7α,7aα)-Hexahydro-2-[3-(trifluoromethyl)phenyl]-4,7-ethano-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione trifluoroacetate (11)



Compound 10 (3.20 g, 7.01 mmol) was dissolved in 100 mL of MeOH and 10% Pd/C Degussa catalyst (2.00 g, cat.) was added. Hydrogen was then introduced via a balloon. After 1 h, the reaction was filtered through Celite and rinsed with MeOH. The volatiles were removed in vacuo and the resulting crude material was purified by reverse phase preparative HPLC to yield 2.50 g (5.70 mmol, 81%) of compound 11 as the TFA salt (white solid). HPLC: 99% at 1.843 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 325.12 $[M+H]^+$.

EXAMPLE 12

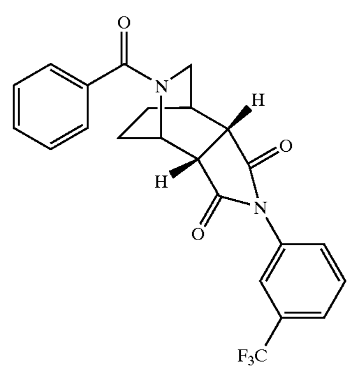
(3aα,4α,7α,7aα)-5-Acetylhexahydro-2-[3-(trifluoromethyl)phenyl]-4,7-ethano-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (12)



Compound 11 (0.10 g, 0.23 mmol) was suspended in THF (5.0 mL) and TEA (0.097 mL, 0.46 mmol) was added resulting in a homogeneous solution. Acetyl chloride (0.033 mL, 0.46 mmol) was then added. After 2 h, the reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with methylene chloride (3×15 mL). The crude material was purified by preparative TLC eluting with chloroform/acetone to give 0.067 g (0.18 mmol, 79%) of compound 12 as a colorless oil. HPLC: 99% at 2.66 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 367.0 $[M+H]^+$.

EXAMPLE 13

(3aα,4α,7α,7aα)-5-Benzoylhexahydro-2-[3-(trifluoromethyl)phenyl]-4,7-ethano-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (13)

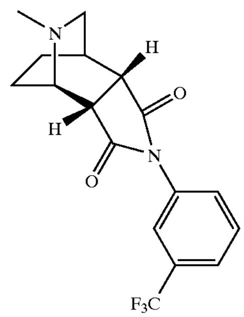


Compound 11 (0.10 g, 0.23 mmol) was suspended in THF (5.0 mL) and TEA (0.097 mL, 0.46 mmol) was added resulting in a homogeneous solution. Benzoyl chloride (0.053 mL, 0.46 mmol) was then added. After 2 h, the reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with methylene chloride (3×15 mL). The crude material was purified by reverse phase preparative HPLC to give 0.020 g (0.047 mmol, 20%) of compound 13 as a white

foam. HPLC: 99% at 3.183 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 429.1 $[M+H]^+$.

EXAMPLE 14

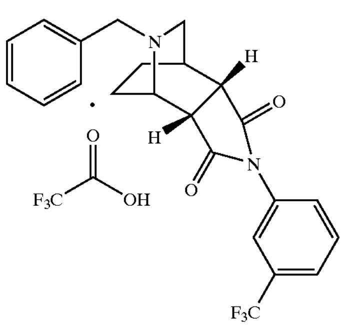
(3aα,4α,7α,7aα)-Hexahydro-5-methyl-2-[3-(trifluoromethyl)phenyl]4,7-ethano-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (14)



Compound 11 (0.10 g, 0.23 μmmol) was suspended in THF (5.0 mL) and TEA (0.097 mL, 0.46 mmol) was added resulting in a homogeneous solution. Dimethyl sulfate (0.043 mL, 0.46 mmol) was added and the reaction was stirred at 25° C. After 14 h, the reaction was concentrated in vacuo and the crude material was purified by preparative TLC eluting with 10% MeOH in methylene chloride to give 0.030 g (0.088 mmol, 39%) of compound 14 as a white solid. HPLC: 100% at 1.797 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 339.21 $[M+H]^+$.

EXAMPLE 15

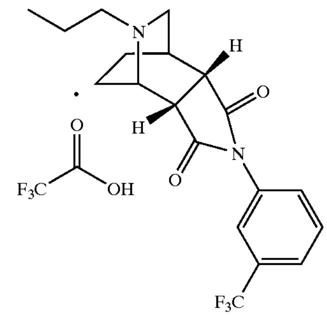
(3aα,4α,7α,7aα)-Hexahydro-5-(phenylmethyl)-2-[3-(trifluoromethyl)phenyl]-4,7-ethano-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione Trifluoroacetate (15)



Compound 11 (0.10 g, 0.23 mmol) was dissolved in DMF (5.0 mL) and K_2CO_3 (0.063 g, 0.46 mmol) was added. Benzyl bromide (0.041 mL, 0.35 mmol) was then added. The reaction was stirred at 25° C. for 1 h, filtered and concentrated in vacuo. The crude material was purified by reverse phase preparative HPLC to give 0.055 g (0.10 mmol, 43%) of compound 15 as a white solid. HPLC: 100% at 2.31 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 415.36 $[M+H]^+$.

EXAMPLE 16

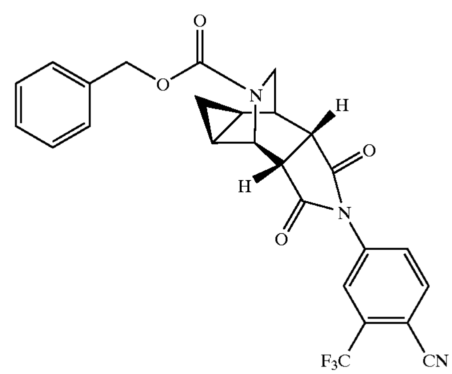
(3aα,4α,7α,7aα-Hexahydro-5-propyl-2-[3-(trifluoromethyl)phenyl]-4,7-ethano-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione Trifluoroacetate (16)



Compound 11 (0.10 g, 0.23 mmol) was dissolved in DMF (5.0 mL) and K_2CO_3 (0.079 g, 0.57 mmol) was added, followed by 1-bromopropane (0.031 mL, 0.34 mmol). The reaction was stirred at 25° C. for 6 h, then filtered and concentrated in vacuo. The crude material was purified by reverse phase preparative HPLC to give 0.070 g (0.15 mmol, 63%) of compound 16 as a white solid. HPLC: 100% at 1.907 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 340.22 $[M+H]^+$.

EXAMPLE 17

(3aα,4α,4aβ,5aβ,6α,6aα)-2-[4-Cyano-3-(trifluoromethyl)phenyl]decahydro-1,3-dioxo-4,6-(iminomethano)cycloprop[f]isoindole-7-carboxylic Acid Phenylmethyl Ester (17)

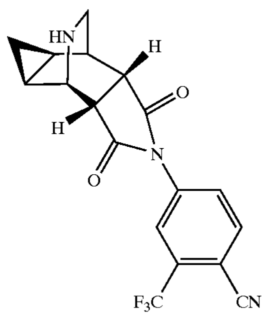


1-Methyl-3-nitro-1-nitrosoguanidine (2.5 g, 17 mmol) was added portionwise to a solution of 40% KOH/H_2O (15 mL) and Et_2O (25 mL) at 0° C. The ether layer turned yellow once addition was complete. After 30+min at 0° C., the ether layer was poured into a solution of (3aα,4α,7α,7aα)-2-[4-cyano-3-(trifluoromethyl)phenyl]-octahydro-1,3-dioxo-4,7-etheno-5H-pyrrolo[3,4-c]pyridine-5-carboxylic acid phenylmethyl ester (0.500 g, 1.09 mmol, prepared as described in Example 10) and $Pd(OAc)_2$ (0.010 g) in THF (10 mL) at 0° C. The reaction was then warmed slowly to 25° C., stirred for 24 h and then filtered through Celite rinsing with THF. The crude material was then purified by flash chromatography on SiO_2 eluting with $MeOH/CH_2Cl_2$ to give 0.34 g (0.69

mmol, 63%) of compound 17 as a white solid and a single isomer. HPLC: 100% at 3.61 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 496.25 $[M+H]^+$.

EXAMPLE 18

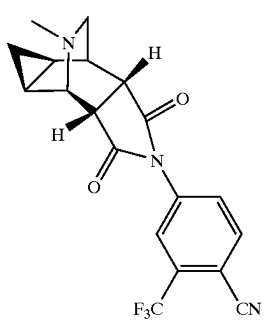
(3aα,4α,4aβ,5aβ,6α,6aα)-4-[Decahydro-1,3-dioxo-4,6-(iminomethano)cyloprop[f]isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (18)



Compound 17 (0.200 g, 0.404 mmol) was dissolved in MeOH (20 mL) and 5% Pd/C (0.200 g) was added. Hydrogen was then introduced via balloon. After 3 h, the reaction was filtered through Celite, rinsed with MeOH and the volatiles were removed in vacuo to yield 0.130 g (0.360 mmol, 89%) compound 18 as a white solid. HPLC: 100% at 1.80 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 362.09 $[M+H]^+$.

EXAMPLE 19

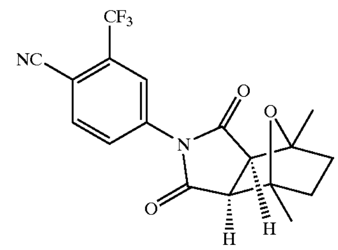
(3aα,4α,4β,5aβ,6α,6aα)-4-[Decahydro-7-methyl-1,3-dioxo-4,6-(iminomethano)cycloprop[f]isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (19)



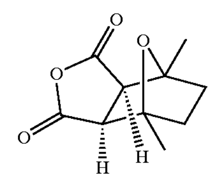
Compound 18 (0.100 g, 0.277 mmol) was dissolved in CH_3CN (2.0 mL). TEA (0.19 mL, 1.4 mmol) and MeI (0.052 mL, 0.83 mmol) were then added and the reaction was stirred at 25° C. for 14 h. The reaction was concentrated under reduced pressure and the crude material was partioned between CH_2Cl_2/water and the aqueous layer was extracted with CH_2Cl_2 (3×15 mL). The combined organics were dried over anhydrous Na_2SO_4. The crude material was purified by flash chromatography eluting with 3% MeOH/CH_2Cl_2 to give 0.030 g (0.080 mmol, 29%) of compound 19 as a light yellow solid. HPLC: 100% at 1.720 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 376.11 $[M+H]^+$.

EXAMPLE 20

(3aα,4β,7β,7aα)-4-(Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (20B)



A. (3aα,4β,7β,7aα)-Hexahydro-4,7-epoxyisobenzofuran-1,3-dione (20A)



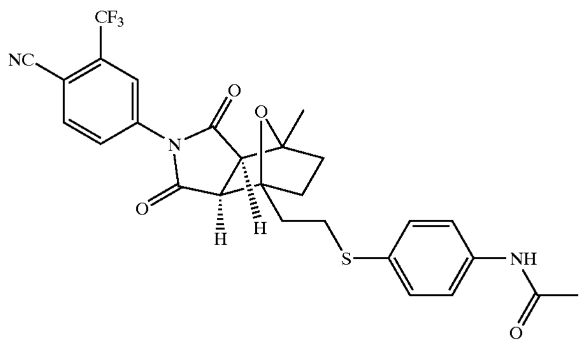
Freshly distilled dimethyl furan (1.60 mL, 15.3 mmol) was dissolved in CH_2Cl_2 (2.0 mL) and maleic anhydride (1.00 g, 10.2 mmol) was added. The reaction was stirred at 25° C. for 16 h and was then concentrated in vacuo to give a yellow solid. This solid was dissolved in ethyl acetate (30 mL) and 10% Pd/C (0.200 g, cat.) was added. Hydrogen was then introduced via a balloon and the reaction stirred for 24 h. The reaction mixture was filtered through Celite rinsing with EtOAc followed by concentration in vacuo to give 1.69 g (8.61 mmol, 84%) of compound 20A as a white solid. 2-Dimensional NOE experiments confirmed the structural assignment to be that of compound 20A.

B. (3aα,4β,7β,7aα)-4-(Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (20B)

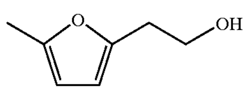
A solution of compound 20A (603 mg, 3.21 mmol), 5-amino-2-cyanobenzotrifluoride (640 mg, 3.44 mmol) and TsOH (10 mg, cat.) in toluene (5 mL) was heated in a sealed tube for 2 days. The reaction mixture was cooled to room temperature and then concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 50% EtOAc/hexanes gave 400 mg (1.10 mmol, 34%) of compound 20B as a white solid. HPLC: 99% at 3.04 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 382.2 $[M+NH_4]^+$.

EXAMPLE 21

(3aα,4β,7β,7aα)-N-[4-[[2-[2-[4-Cyano-3-(trifluoromethyl)phenyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethyl]thio]phenyl]acetamide (21E)

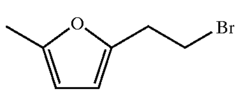


A. 5-Methyl-2-furanethanol (21A)



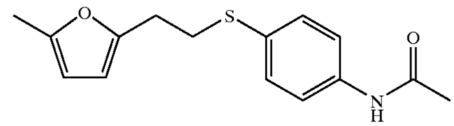
A solution of n-BuLi (83.0 mL, 133 mmol, 1.6 M in hexanes) was added to a stirred solution of 2-methylfuran (10.0 mL, 111 mmol) in THF (85 mL) at 0° C. under inert atmosphere. The reaction mixture was stirred for 4 h at room temperature then cooled to 0° C. Ethylene oxide (8.30 mL, 166 mmol) was added dropwise and the reaction mixture was allowed to warm to room temperature overnight. After quenching with saturated aqueous NH_4Cl, the resulting layers were separated and the aqueous layer was extracted with Et_2O (2×250 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Distillation at atmospheric pressure (170–185° C.) gave 10.1 g (80.3 mmol, 72%) of compound 21A as a light yellow oil.

B. 2-(2-Bromoethyl)-5-methylfuran (21B)



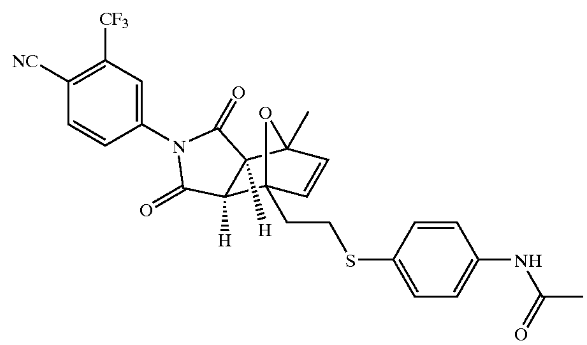
Ph_3Br_2 (3.68 g, 8.72 mmol) was added to a solution of compound 21A (1.00 g, 7.93 mmol) in DMF (8 mL) and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was added to H_2O and extracted with EtOAc (3×). The combined organic layers were washed with H_2O (2×), dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 10% EtOAc/hexanes gave 0.507 g (2.68 mmol, 34%) of compound 21B.

C. N-[4-[[2-(5-Methyl-2-furanyl)ethyl]thio]phenyl]acetamide (21C)



To a solution of 4-acetamidothiophenol (442 mg, 2.64 mmol) in THF (1 mL) at 0° C. under inert atmosphere was added a solution of n-BuLi (2.00 mL, 3.17 mmol, 1.6 M in hexanes) in THF (1 mL). The reaction solution was stirred at room temperature for 10 min then a solution of compound 21B (500 mg, 2.64 mmol) in THF (3 mL) was added. After the starting material was consumed (as determined by TLC), the reaction was quenched with H_2O and the mixture was extracted with EtOAc (2×), dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 50% EtOAc/hexanes gave 0.644 g (2.34 mmol, 88%) of compound 21C. MS (ESI): m/z 276.09 $[M+H]^+$.

D. (3aα,4β,7β,7aα)-N-[4-[[2-[2-[4-Cyano-3-(trifluoromethyl)phenyl]-1,2,3,3a,7,7a-hexahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethyl]thio]phenyl]acetamide (21D)



A solution of compound 21C (195 mg, 0.708 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (377 mg, 1.416 mmol, prepared as described for Example 1B) in CH_2Cl_2 (1.5 mL) was stirred at room temperature for two days. The reaction mixture was concentrated under reduced pressure to yield compound 21D as determined by NMR analysis. Compound 21D was used directly in the next step without purification.

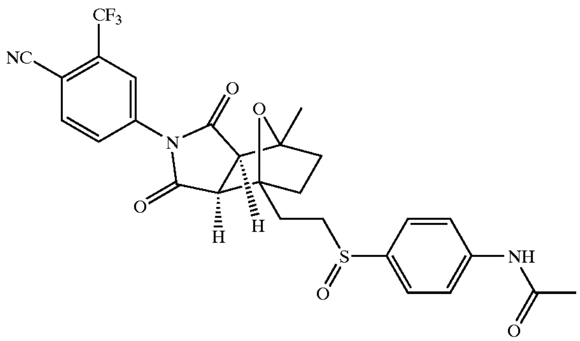
E. (3aα,4β,7β,7aα)-N-[4-[[2-[2-[4-Cyano-3-(trifluoromethyl)phenyl]-octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethyl]thio]-phenyl]acetamide (21E)

A solution of crude compound 21D (0.708 mmol) and 10% Pd/C (200 mg) in MeOH (20 mL) was stirred under a hydrogen atmosphere overnight. Purification by reverse phase HPLC [34.4 min (retention time) (YMC S5 ODS column 20×250 mm, 0–100% aqueous methanol over 30 minutes containing 0.1% TFA, 10 mL/min, monitoring at 220 nm)] followed by flash chromatography on silica gel eluting with 1% $MeOH/CH_2Cl_2$ gave 29 mg (0.053 mmol, 7.5%) of compound 21E as a yellow powder. HPLC: 99% at 3.44 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing

0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 544.01 $[M+H]^+$.

EXAMPLE 22

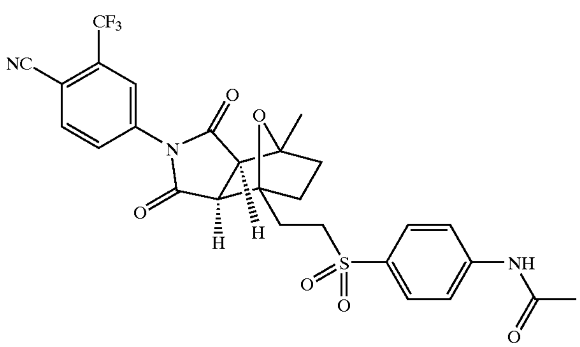
(3aα,4β,7β,7aα)-N-[4-[[2-[2-[4-Cyano-3-(trifluoromethyl)phenyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethyl]sulfinyl]phenyl]acetamide (22)



mCPBA (12 mg, 0.050 mmol) was added portionwise to a solution of crude compound 21E (65 mg, 0.12 mmol) in CH_2Cl_2 (6 mL) until the starting material was consumed. Purification by reverse phase HPLC [30.5 min (retention time) (YMC S5 ODS column 30×250 mm, 0–100% aqueous methanol over 30 minutes containing 0.1% TFA, 25 mL/min, monitoring at 220 nm)] gave 27.5 mg (0.0491 mmol, 41%) of compound 22 as a tan solid (~1:1 mixture of diastereomers). HPLC: 96% at 2.88 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 559.97 $[M+H]^+$.

EXAMPLE 23

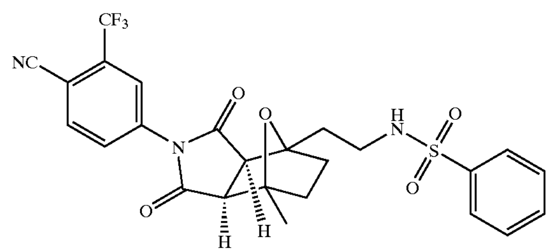
(3aα,4β,7β,7aα)-N-[4-[[2-[2-1-[4-Cyano-3-(trifluoromethyl)phenyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethyl]sulfonyl]phenyl]acetamide (23)

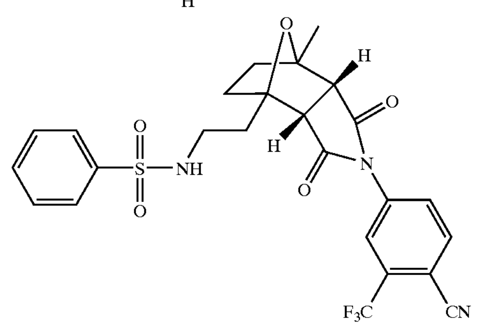


mCPBA (26 mg, 0.11 mmol) was added to a solution of compound 21E (19 mg, 0.035 mmol) in CH_2Cl_2 (6 mL) and the reaction was stirred at rt until starting material and the intermediate sulfoxide (compound 22) were consumed as was apparent by TLC. Purification by reverse phase preparative HPLC [53.3 min (retention time) (YMC S5 ODS column 30×250 mm, 0–70% aqueous methanol over 45 minutes containing 0.1% TFA, 25 mL/min, monitoring at 220 nm)] gave 8.0 mg mg (0.014 mmol, 40%) of compound 23 as a white solid. HPLC: 99% at 2.94 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 575.95 $[M+H]^+$.

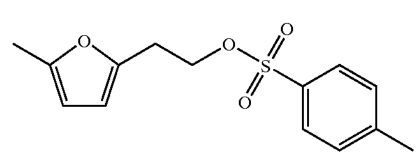
EXAMPLE 24

(3aα,4β,7β,7aα)- and (3aα,4α,7α,7aα)-N-[2-[2-[4-Cyano-3-(trifluoromethyl)phenyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethyl]benzenesulfonamide (24Ci and 24Cii, Respectively)





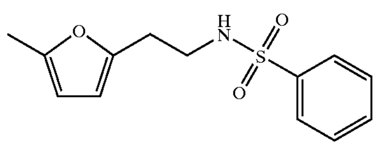
A. 5-Methyl-2-furanethanol 4-methylbenzenesulfonate (24A)



4-Methylbenzenesulfonyl chloride (907 mg, 4.76 mmol) was added to a solution of compound 21A (500 mg, 3.96 mmol) in 6 mL of dry pyridine. The reaction was stirred at room temperature for 4 h and then quenched with ice. The

reaction mixture was extracted with CH_2Cl_2 and the combined organic layers were washed with saturated aqueous sodium bicarbonate and water, dried and concentrated under reduced pressure to give 900 mg (81%) of compound 24A as a yellow oil.

B. N-[2-(5-Methyl-2-furanyl)ethyl] benzenesulfonamide (24B)



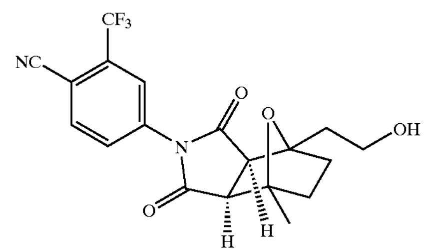
Benzenesulfonamide (157 mg, 1.00 mmol) was added to a 10% aqueous solution of sodium hydroxide (0.40 mL, 1.0 mmol). A solution of compound 24A (280 mg, 1.00 mmol) in acetone (1 mL) was then added. The reaction mixture was heated at 90° C. for 8 h then cooled to room temperature. Ice was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were washed with water, dried and concentrated under reduced pressure. Purification by flash chromatography on silica gel, eluting with CH_2Cl_2 gave 60 mg (0.23 mmol, 23%) of compound 24B as yellow oil.

C. (3aα,4β,7β,7aα)- and (3aα,4α,7α,7aα)-N-[2-[2-[4-Cyano-3-(trifluoromethyl)phenyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethyl] benzenesulfonamide (24Ci and 24Cii, Respectively)

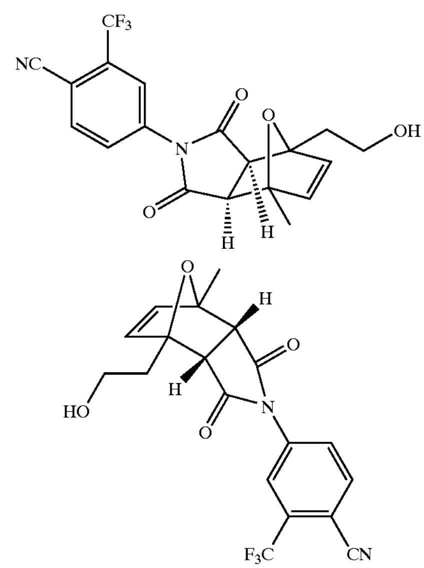
4-(2,5-Dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (129 mg, 0.485 mmol, prepared as described in Example 1B) was added to a solution of compound 24B (60 mg, 0.23 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred at room temperature for 2 days, concentrated under reduced pressure and purified by flash chromatography on silica gel, eluting with 70% EtOAc/hexanes, to give 20 mg (0.038 mmol, 16%) of the unsaturated Diels-Alder product. The unsaturated product (20 mg) was immediately dissolved in ethanol (2 mL) and 10% Pd/C (10 mg, cat.) was added. The solution was stirred at room temperature overnight under a hydrogen atmosphere. The mixture was filtered and the filtrate was concentrated under reduced pressure. Purification by reverse phase preparative HPLC gave 7.0 mg (0.013 mmol, 34%) of compound 24Ci and 2.0 mg (0.0037 mmol, 10%) of compound 24Cii. Compound 24Ci: HPLC: 96% at 3.17 min (retention time) (YMC ODSA S5 C18 4.6×50 mm, 10%–90% aqueous methanol over 4 min gradient with 0.1% TFA, monitoring at 220 nm). MS (ES): m/z: 533.99 $[M+H]^+$. Compound 24Cii: HPLC: 99% at 38.95 min (retention time) (YMC ODS S5 20×250 mm, 10%–90% aqueous methanol over 40 min gradient with 0.1% TFA, monitoring at 220 nm). MS (ES): m/z 533.99 $[M+H]^+$.

EXAMPLE 25

(3aα,4β,7β,7aα)-4-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (25B)



A. (3aα,4β,7β,7aα)- and (3aα,4α,7α,7aα)-4-[1,3,3a,4,7,7a-Hexahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (25Ai and 25Aii, Respectively)



A solution of compound 21A (252 mg, 2.00 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (798 mg, 3.00 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature for 2 days. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 65% EtOAc/hexanes gave 217 mg of pure compound 25Ai, 73 mg of pure compound 25Aii and 310 mg of a mixture of both compound 25Ai and 25Aii. All three fractions were isolated as white solids with a total isolated yield of 600 mg (1.53 mmol, 76.5%). Compound 25Ai: HPLC: 90% at 2.56 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). Compound 25Aii: HPLC: 90% at 2.56 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

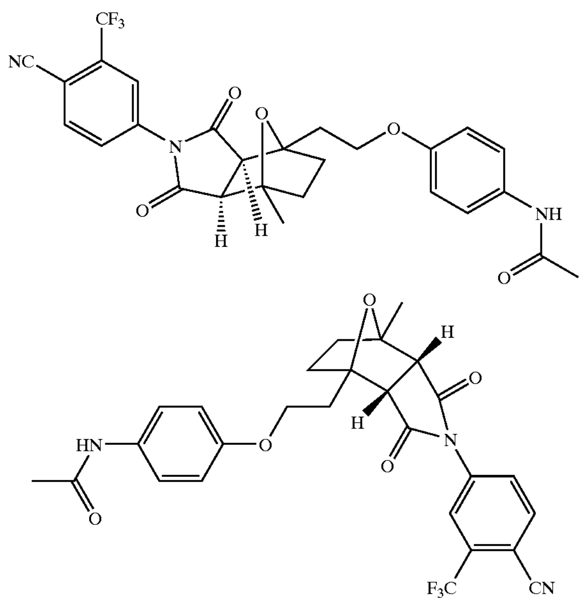
B. (3aα,4β,7β,7aα)-4-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (25B)

A solution of compound 25Ai (0.20 g, 0.51 mmol) and 10% Pd/C (43 mg, cat.) in EtOH (12 mL) was stirred under

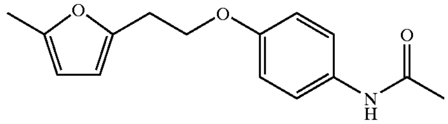
a hydrogen atmosphere at room temperature for 2 h. The reaction mixture was filtered through Celite and concentrated under reduced pressure to give 0.20 g (0.51 mmol, 100%) of compound 25B as a white solid. HPLC: 95% at 2.59 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 394.97 $[M+H]^+$.

EXAMPLE 26

(3aα,4β,7β,7aα)- and (3aα,4α,7α,7aα)-N-[4-[2-[2-[4-Cyano-3-(trifluoromethyl)phenyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl) ethoxy]phenyl]acetamide (26Ci and 26Cii, Respectively)

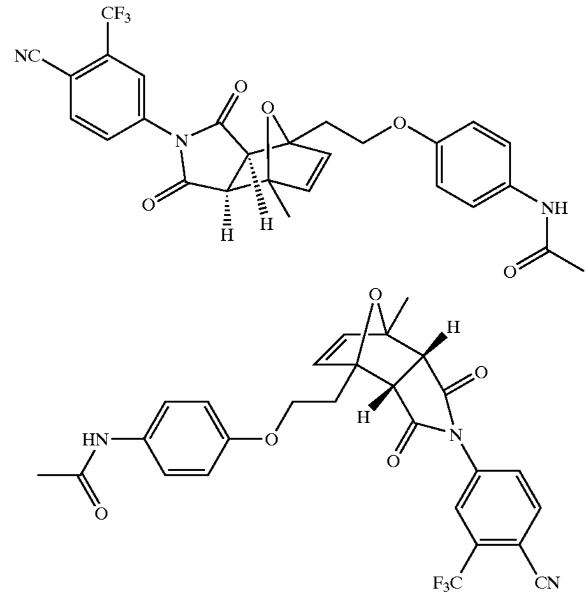


A. 2-[4-[2-(5-Methyl-2-furanyl)ethoxy]phenyl] acetamide (26A)



Triphenylphosphine (681 mg, 2.60 mmol) was added to a solution of compound 21A (252 mg, 2.00 mmol) and 4-acetamidophenol (302 mg, 2.00 mmol) in CH_2Cl_2 (4 mL). THF (5 mL) was added to make the reaction mixture homogeneous and the mixture was then cooled to 0° C. DEAD (0.41 mL, 2.6 mmol) was added dropwise and the reaction mixture was stirred at room temperature overnight, then concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 60% EtOAc/hexanes followed by reverse phase preparative HPLC gave 270 mg (1.04 mmol, 52%) of compound 26A as a light brown solid. MS (ESI): m/z 260.09 $[M+H]^+$.

B. (3aα,4β,7β,7aα)- and (3aα,4α,7α,7aα)-N-[4-[2-[2-[4-Cyano-3-(trifluoromethyl)phenyl]-1,2,3,3a,7,7a-hexahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy]phenyl]acetamide (26Bi and 26Bii, Respectively)



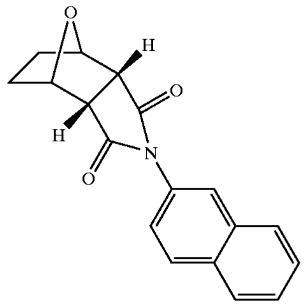
A solution of compound 26A (40 mg, 0.15 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (88 mg, 0.31 mmol) in CH_2Cl_2 (2 mL) was stirred at room temperature for 2 days. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 75% EtOAc/hexanes gave 55 mg (0.105 mmol, 68%) of a 5:1 mixture of compounds 26Bi and 26Bii as a white solid, which was used directly in the next step. HPLC: 90% at 3.28 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

C. (3aα,4β,7β,7aα)- and (3aα,4α,7α,7aα)-N-[4-[2-[2-[4-Cyano-3-(trifluoromethyl)phenyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl] ethoxy]phenyl]acetamide (26Ci and 26Cii, Respectively)

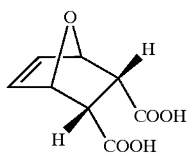
A solution of a mixture of compounds 26Bi and 26Bii (55 mg, 0.105 mmol) and 10% Pd/C (12 mg, cat.) in EtOH (3 mL) was stirred under a hydrogen atmosphere at room temperature overnight. The reaction mixture was filtered through Celite and concentrated under reduced pressure to give 50 mg of crude product. Purification by flash chromatography on silica gel eluting with 70% EtOAc/hexanes gave 18 mg (0.034 mmol, 32%) of compound 26Ci [HPLC: 96% at 3.33 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 528.01 $[M+H]^+$]; and 2.3 mg (0.0044 mmols, 4%) of an 85:15 mixture of 26Cii and 26Ci respectively as determined by ^1H NMR. HPLC: 90% at 3.35 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 528.12 $[M+H]^+$.

EXAMPLE 27

(3aα,4α,7α,7aα)-Hexahydro-2-(2-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione (27D)

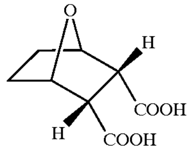


A. (endo, endo)-7-Oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic Acid (27A)



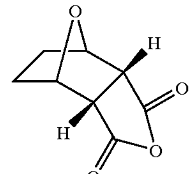
Compounds 27A, 27B and 27C were synthesized in accordance with the approaches described in Sprague et al. *J. Med. Chem.* 28, 1580–1590 (1985). A mixture of furan (100 mL, 1.38 mol) and maleic acid (160 g, 1.38 mol) in H_2O (340 mL) was stirred at room temperature for 5 days. The mixture was placed in a separatory funnel and the aqueous layer was separated from the layer containing the unreacted furan. The aqueous layer was treated with charcoal, filtered through Celite and placed in the refrigerator. The desired product crystallized from solution upon seeding, was filtered, washed with cold water and dried over P_2O_5 to give 70 g (0.38 mol, 28%) of compound 27A as a white solid.

B. (endo, endo)-7-Oxabicyclo[2.2.1]heptane-2,3-dicarboxylic Acid (27B)



To a solution of compound 27A (69.0 g, 0.375 mol) in EtOH (700 mL) was added 10% Pd/C (4.5 g, cat.) and the mixture was shaken under a hydrogen atmosphere at 55 psi until gas uptake ceased. The mixture was filtered through Celite and concentrated in vacuo to give 66.0 g (0.355 mol, 95%) of compound 27B as a white solid.

C. (3aα,4α,7α,7aα)-Hexahydro-4,7-epoxyisobenzofuran-1,3-dione (27C)



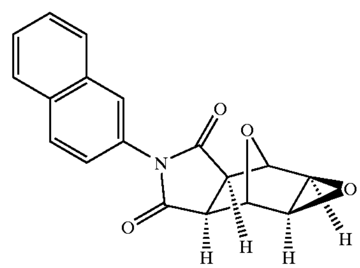
A solution of compound 27B (66.0 g, 355 mol) in acetyl chloride (300 mL) was refluxed for 1 h. The reaction solution was concentrated in vacuo and the resulting residue was recrystallized from benzene to give 49.2 g (0.292 mol, 82%) of compound 27C as a white solid (>99% endo by ^{1}H NMR).

D. (3aα,4α,7α,7aα)-Hexahydro-2-(2-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione (27D)

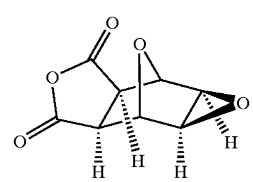
Compound 27C (45 mg, 0.30 mmol) was combined with 2-aminonaphthalene (47 mg, 0.33 mmol) in acetic acid (1 mL) and heated at 115° C. overnight. After the reaction was cooled to rt, a drop of water was added, and the resulting precipitate was filtered. The material was washed with methanol and dried to provide 65.7 mg (0.224 mmol, 74.7%) of compound 27D as a white crystalline solid. HPLC: 99% at 2.68 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 294.0 $[M+H]^+$.

EXAMPLE 28

(1aα,2β,2aα,5aα,6β,6aα)-Hexahydro-4-(2-naphthalenyl)-2,6-epoxy-3H-oxireno[f]isoindole-3,5(4H)-dione (28B)



A. (1aα,2β,2aα,5aα,6β,6aα)-Tetrahydro-2,6-epoxyoxireno[f]isobenzofuran-3,5(2aH,5aH)-dione (28A)



As described in Yur'ev et al. *J. Gen. Chem. U.S.S.R.* (*Engl. Transl.*) 31, 772–775 (1961), a solution of exo-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride (5.00

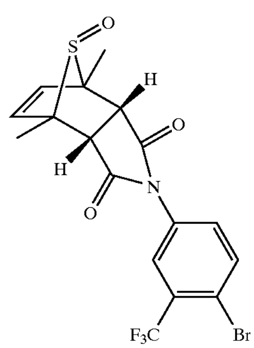
g, 30.1 mmol), formic acid (10 mL) and hydrogen peroxide (6 mL) was stirred at room temperature. After 30 min, the reaction was placed in an ice bath (it became exothermic along with gas evolution) and was allowed to warm to room temperature slowly. After stirring overnight, the resulting precipitate was collected by filtration and washed with glacial acetic acid and dried to yield 3.02 g of a white powder. The crude solid was boiled in acetyl chloride (100 mL) for 10 hours and the mixture was concentrated to ~20 mL under reduced pressure. The resulting precipitate was filtered, washed with dioxanes and dried to give 2.37 g (13.0 mmol, 43%) of compound 28A as a white powder.

B. (1aα,2β,2aα,5aα,6β,6aα)-Hexahydro-4-(2-naphthalenyl)-2,6-epoxy-3H-oxireno[f]isoindole-3,5(4H)-dione (28B)

Compound 28A (100 mg, 0.520 mmol) was combined with 2-aminonaphthalene (62.1 mg, 0.434 mmol) in acetic acid (2 mL) and heated at 115° C. overnight. After the reaction was allowed to cool to rt, water was added, and the resulting precipitate was filtered. The material was washed sequentially with aqueous K_2CO_3 and water and then dried in a vacuum oven to provide 113.7 mg (0.371 mmol, 85.5%) of compound 28B as an off-white crystalline solid. HPLC: 99% at 1.76 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 308.0 $[M+H]^+$.

EXAMPLE 29

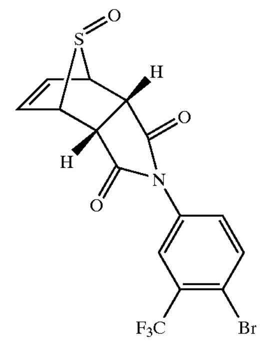
(3aα,4α,7α,7aα)-2-[4-Bromo-3-(trifluoromethyl)phenyl]-3a,4,7,7a-tetrahydro-4,7-dimethyl-4,7-epithio-1H-isoindole-1,3(2H)-dione 8-oxide (29)



2,5-Dimethylthiophene (0.048 mL, 0.42 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (0.290 g, 0.625 mmol, prepared as described for Example 1B) were dissolved in CH_2Cl_2 (8.0 mL) and cooled to −20° C. $BF_3.Et_2O$ (0.412 mL, 3.36 mmol) was added slowly followed by addition of mCPBA (~50%, 0.29 g, 0.84 mmol). After 2 h at −20° C., the reaction mixture was poured into saturated aq. $NaHCO_3$ and extracted with CH_2Cl_2 (3×20 mL) and the organics dried over anhydrous Na_2SO_4. The crude product was purified by flash chromatography on SiO_2 eluting with 5%–10%–20% EtOAc in CH_2Cl_2 to give 0.119 g (0.265 mmol, 63%) of compound 29 as a white solid. HPLC: 91% at 3.303 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 480.2 $[M+H]^+$.

EXAMPLE 30

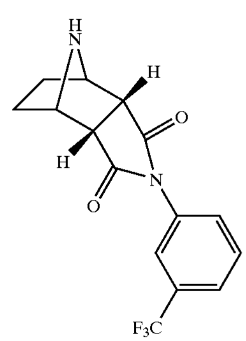
(3aα,4α,7α,7aα)-2-[4-Bromo-3-(trifluoromethyl)phenyl]-3a,4,7,7a-tetrahydro-4,7-epithio-1H-isoindole-1,3(2H)-dione 8-oxide (30)



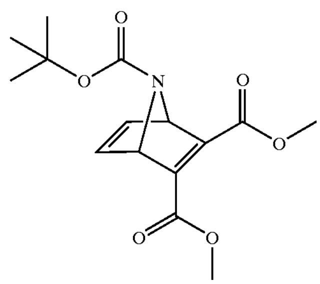
Thiophene (0.375 mL, 4.69 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (0.100 g, 0.313 mmol, prepared as described for Example 1B) were dissolved in CH_2Cl_2 (50 mL), mCPBA (~50%, 1.62 g, 4.69 mmol) was added and the resulting mixture was stirred at 25° C. for 3 h. Triphenylphosphine (2.0 g) was then added. After 15 min, the volatiles were removed in vacuo and the resulting residue was dissolved in CH_2Cl_2 (200 mL) and washed with saturated aq. $NaHCO_3$ (3×50 mL) and dried over Na_2SO_4. The crude material was then purified by flash chromatography on SiO_2 eluting with 1%–3%–5% methanol in CH_2Cl_2 to give 0.059 g (0.14 mmol, 45%) compound 30 as a white powder. NMR and LC analysis showed a single diastereomer. HPLC: 100% at 3.437 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 443.2 $[M+H]^+$.

EXAMPLE 31

(3aα,4α,7α,7aα)-Hexahydro-2-[3-(trifluoromethyl)phenyl]-4,7-imino-1H-isoindole-1,3(2H)-dione (31D)

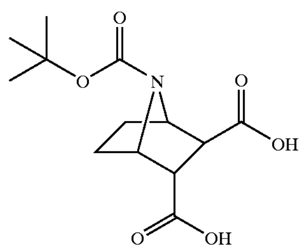


A. 7-Azabicyclo[2.2.1]hepta-2,5-diene-2,3,7-tricarboxylic Acid 2,3-dimethyl 7-(1,1-dimethylethyl)ester (31A)



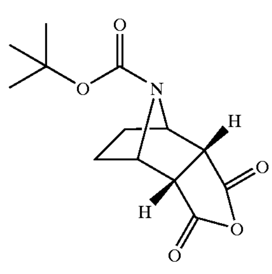
Freshly distilled acetylenedicarboxylic acid dimethyl ester (6.7 mL, 54 mmol) and N-(tert-butyloxycarbonyl)-1H-pyrrole (9.0 mL, 54 mmol) were combined and heated at 120° C. for 3 h. Purification by flash chromatography on SiO_2 eluting with EtOAc/CH_2Cl_2 gave 8.3 g (27 mmol, 50%) of compound 31A as a yellow solid.

B. (exo,endo)-7-Azabicyclo[2.2.1]hept-2,5-diene-2,3,7-tricarboxylic Acid 7-(1,1-dimethylethyl)ester (31B)



Compound 31A (1.0 g, 3.5 mmol) was dissolved in MeOH (2.0 mL) and aq. KOH (1 g in 5 mL H_2O) was added. The reaction was heated at 50° C. for 1 h. The reaction was then cooled to 25° C. and 10% Pd/C (0.5 g, cat.) was added and the mixture was placed in a Parr apparatus for 14 h at 25° C. The reaction was then filtered through Celite rinsing with water. The aqueous solution was acidified to pH 2 by addition of 1 N HCl and then extracted with EtOAc (2×100 mL). Concentration of the organics gave the compound 31B as a pale yellow solid.

C. (3aα,4α,7α,7aα)-Hexahydro-1,3-dioxo-4,7-iminoisobenzofuran-8-carboxylic Acid 1,1-dimethylethyl Ester (31C)



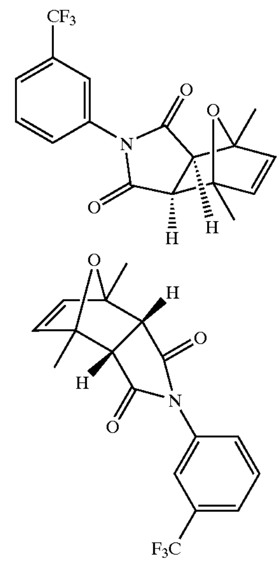
Crude compound, 31B, was heated to 120° C. in vacuo in a sublimation chamber, resulting in sublimation of 0.051 g (0.19 mmol, 5.4%) of compound 31C as a white solid, which was collected directly and used in the next step without further purification.

D. (3aα,4α,7α,7aα)-Hexahydro-2-[3-(trifluoromethyl)phenyl]-4,7-imino-1H-isoindole-1,3(2H)-dione (31D)

Compound 31C (0.050 g, 0.19 mmol) and the 1-amino-3-(trifluoromethyl)benzene (0.03.0 g, 0.19 mmol) were dissolved in AcOH (2.5 mL) and heated at 115° C. for 4.5 h. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ and the mixture was extracted with methylene chloride (3×15 mL). The crude material was purified by reverse phase preparative HPLC to give 0.030 g (0.097 mmol, 51%)of compound 31D as a white solid. HPLC: 99% at 2.33 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 311.15 $[M+H]^+$.

EXAMPLE 32

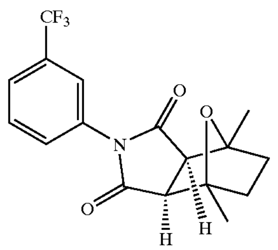
(3aα,4β,7β,7aα)- and (3aα,4α,7α,7aα)-3a,4,7,7a-Tetrahydro-4,7-dimethyl-2-[3-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione (32i and 32ii, Respectively)



Freshly distilled 2,5-dimethylfuran (0.32 mL, 2.6 mmol) was dissolved in CH_2Cl_2 (2.0 mL) and 1-[3-(trifluoromethyl)phenyl]-1H-pyrrole-2,5-dione (0.5 g, 2.5 mmol, prepared as described in Example 1B) was added. The reaction was stirred at 25° C. for 16 h and was then concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 0.5% MeOH/CH_2Cl_2 gave 250 mg (0.741 mmol, 30%) of compound 32i, and 50 mg (0.15 mmol, 6%) of compound 32ii as white solids. Compound 32i: HPLC: 98% at 3.080 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 338.30 $[M+H]^+$. Compound 32ii: HPLC: 92% at 3.047 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm), MS (ES): m/z: 338.15 $[M+H]^+$.

EXAMPLE 33

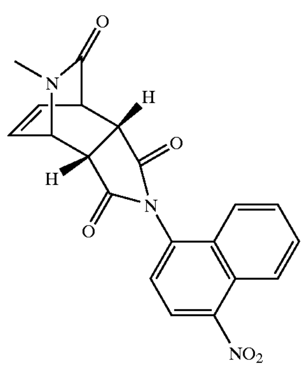
(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-[3-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione (33)



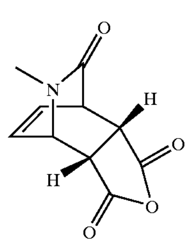
Compound 32i (0.080 g, 0.24 mmol) was dissolved in EtOAc (2 mL) and EtOH (1 mL) and 10% Pd/C (0.050 g, cat.) was added. Hydrogen was then introduced by a balloon and the reaction was stirred for 24 h. The mixture was filtered through Celite, rinsed with EtOAc and concentrated in vacuo to give 0.075 g (0.22 mmol, 93%) of compound 33 as a white solid. No further purification was needed. HPLC: 90% at 3.233 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 340.40 $[M+H]^+$.

EXAMPLE 34

(3aα,4β,7β,7aα)-Tetrahydro-5-methyl-2-(4-nitro-1-naphthalenyl)-4,7-etheno-1H-pyrrolo[3,4-c]pyridine-1,3,6(2H,5H)-trione (34B)



A. 4,5,7,7a-Tetrahydro-5-methyl-4,7-ethenofuro[3,4-c]pyridine-1,3,6(3a H)-trione (34A)



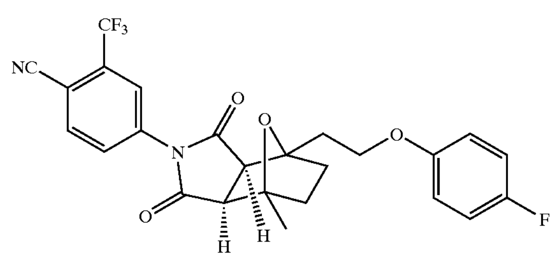
Compound 34A was synthesized by a modification of the methods described by Tomisawa et al. *Heterocycles* 6, 1765–1766 (1977) & *Tetrahedron Lett.* 29, 2465–2468 (1969). Maleic anhydride (2.00 g, 20.4 mmol) and 1-methyl-2-pyridone (2.22 g, 20.4 mmol) were suspended in 30 mL of anhydrous toluene. The reaction vessel was fitted with a Dean Stark trap and refluxed for 48 hours. The dark colored solution was allowed to cool to rt and then the volatiles were removed in vacuo. The resulting brown paste (4 g) was dissolved in 10 mL of boiling toluene and the hot solution was filtered under a nitrogen flow to remove particulates. On standing at 25° C. the desired product precipitated from solution. The solid was isolated by filtration and washed with cold toluene to give 1.0 g (4.8 mmol, 24%) of compound 34A, which was used without further purification.

B. (3aα,4α,7α,7aα)-Tetrahydro-5-methyl-2-(4-nitro-1-naphthalenyl)-4,7-etheno-1H-pyrrolo[3,4-c]pyridine-1,3,6(2H,5H)-trione (34B)

1-Amino-4-nitronaphthalene (0.094 g, 0.50 mmol) and compound 34A (0.130 g, 0.627 mmol) were dissolved in AcOH (2.0 mL) and heated at 110° C. for 11 h. The reaction was then cooled to 25° C. and poured into cold saturated aqueous K_2CO_3 and stirred vigorously for 10 min. The solution was filtered and rinsed with water. The resulting filtrate was concentrated in vacuo and purified by flash chromatography on silica gel eluting with 4:6 EtOAc/hexanes to give 0.172 g (0.456 mmol, 91%) of compound 34B as a white solid. HPLC: 92% at 2.472 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 378.29 $[M+H]^+$.

EXAMPLE 35

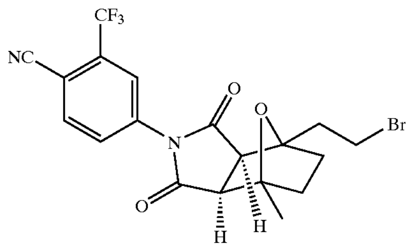
(3aα,4β,7β,7aα)-4-[4-[2-(4-Fluorophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (35)



DEAD (0.060 mL, 0.38 mmol) was added to a solution of triphenylphosphine (100 mg, 0.380 mmol) in THF (1.3 mL) at room temperature under an inert atmosphere. After stirring for 10 min, 4-fluorophenol (43 mg, 0.380 mmol) was added in one portion. The reaction mixture was stirred for 5 min, compound 25B (100 mg, 0.254 mmol) was added and stirring was continued for 3.5 h. Purification by flash chromatography on silica gel eluting with 50% EtOAc/hexanes followed by reverse phase preparative HPLC [11.93 min (retention time) (YMC S5 ODS column 20×100 mm, 0–100% aqueous methanol over 10 minutes containing 0.1% TFA, 20 mL/min, monitoring at 220 nm)] gave 72 mg (58%) of compound 35 as a solid. HPLC: 99% at 3.74 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 487.1 $[M-H]^-$.

EXAMPLE 36

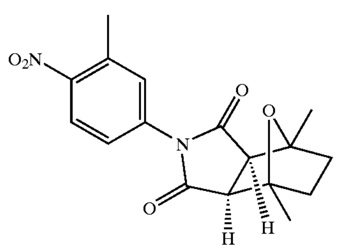
(3aα,4β,7β,7aα)-4-[4-(2-Bromoethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (36)



A solution of 25B (495 mg, 1.26 mmol) and pyridine (100 μL, 1.26 mmol) in CH_2Cl_2 (2 mL) was added to a solution of Ph_3PBr_2 (636 mg, 1.51 mmol) in CH_2Cl_2 (2 mL) at 0° C. The reaction mixture was stirred at room temperature for 3 hr, then the solvent was removed under reduced pressure. The resulting residue was washed 2× with 10 mL portions of EtOAc-hexane (6:4) and the combined washings were purified by flash chromatography on silica gel eluting with 60% EtOAc/hexane to give 390 mg (0.853 mmol, 67.7%) of compound 36 as a white solid. HPLC: 99% at 3.51 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 456.7 $[M-H]^-$.

EXAMPLE 37

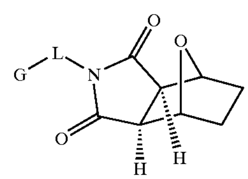
(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-(3-methyl-4-nitrophenyl)-4,7-epoxy-1H-isoindole-1,3 (2H)-dione (37)



A combination of 4-nitro-3-methylaniline (0.050 g, 0.33 mmol), compound 20A (0.083 g, 0.43 mmol), TEA (0.2 mL), $MgSO_4$ (0.075 g) and toluene (0.8 mL) were combined in a sealed tube and the mixture was heated at 120° C. for 14 h. After cooling to 25° C., the reaction was filtered, rinsed with CH_2Cl_2 and concentrated under reduced pressure. The crude product was purified by preparative TLC on SiO_2 eluting with CH_2Cl_2 to give 0.075 g (0.23 mmol, 69%) of compound 37 as a pale yellow solid. HPLC: 100% at 2.733 min (retention time) (YMC S5 ODS column, 4.6×50 mm; 10–90% $MeOH/H_2O$ gradient,+0.1% TFA; 4 mL/min, 220 nm detection). MS (ES): m/z 348.2 $[M+NH_4]^+$.

EXAMPLES 38 TO 121

Additional compounds of the present invention were prepared by procedures analogous to those described above. The compounds of Examples 38 to 121 have the following structure (L is a bond):



where G, the compound name, retention time, molecular mass, and the procedure employed, are set forth in Table 2. The chromatography techniques used to determine the compound retention times of Table 2 are as follows: LCMS= YMC S5 ODS column, 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 4 minutes containing 0.1% TFA; 4 mL/min, monitoring at 220 nm. The molecular mass of the compounds listed in Table 2, where provided, were determined by MS (ES) by the formula m/z.

TABLE 2

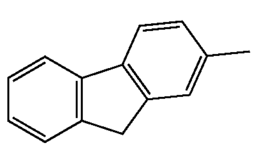
Ex. No.	G	Compound Name	Retention Time (Min.)/ Molecular Mass	Pro. of Ex.
38		(3aα,4β,7β,7aα)-2-(2-Fluorenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.72 LCMS/ 332.20 $[M + H]^+$	8

TABLE 2-continued

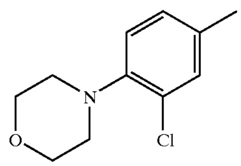
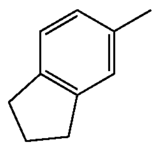
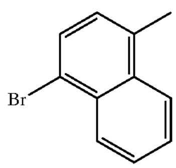
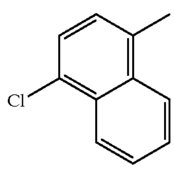
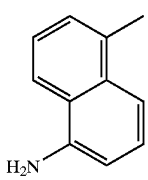
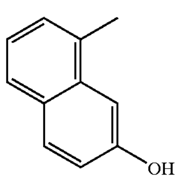
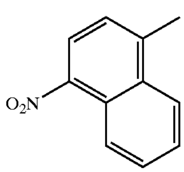
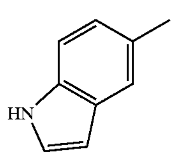
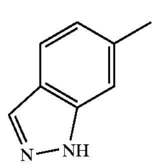
Ex. No.	G	Compound Name	Retention Time (Min.)/ Molecular Mass	Pro. of Ex.
39		(3aα,4β,7β,7aα)-2-[3-Chloro-4-(4-morpholinyl)phenyl]hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.20 LCMS/ 363.20 $[M + H]^+$	8
40		(3aα,4β,7β,7aα)-2-(2,3-Dihydro-1H-inden-5-yl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.26 LCMS/ 284.22 $[M + H]^+$	8
41		(3aα,4β,7β,7aα)-2-(4-Bromo-1-naphthalenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.73 LCMS/ 404.11 $[M + CH_3OH + H]^+$	8
42		(3aα,4β,7β,7aα)-2-(4-Chloro-1-naphthalenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.63 LCMS/ 328.14 $[M + H]^+$	8
43		(3aα,4β,7β,7aα)-2-(5-Amino-1-naphthalenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3-(2H)-dione	1.64 LCMS/	8
44		(3aα,4β,7β,7aα)-Hexahydro-2-(7-hydroxy-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.54 LCMS/ 308.23 $[M - H]^-$	8
45		(3aα,4β,7β,7aα)-Hexahydro-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.117 LCMS/ 404.11 $[M + CH_3OH + H]^+$	8
46		(3aα,4β,7β,7aα)-Hexahydro-2-(1H-indol-5-yl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.39 LCMS/ 282.23 $[M + H]^+$	8
47		(3aα,4β,7β,7aα)-Hexahydro-2-(1H-indazol-6-yl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.35 LCMS/ 282.23 $[M - H]^+$	8

TABLE 2-continued

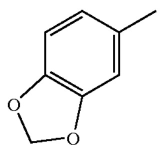
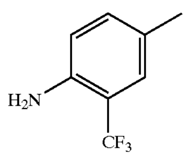
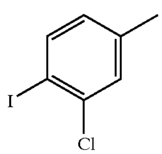
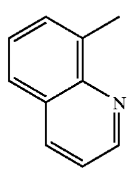
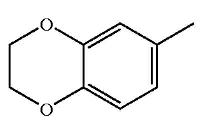
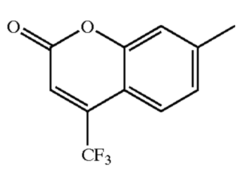
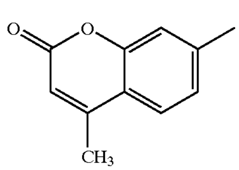
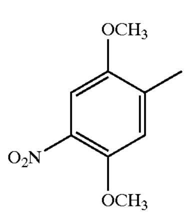
Ex. No.	G	Compound Name	Retention Time (Min.)/ Molecular Mass	Pro. of Ex.
48		(3aα,4β,7β,7aα)-2-(1,3-Benzodioxol-5-yl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.47 LCMS/ 288.20 $[M + H]^+$	8
49		(3aα,4β,7β,7aα)-2-[4-Amino-3-(trifluoromethyl)phenyl]hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.71 LCMS/ 327.20 $[M + H]^+$	8
50		(3aα,4β,7β,7aα)-2-(3-Chloro-4-iodophenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.70 LCMS/ 435.2 $[M + CH_3OH]^+$	8
51		(3aα,4β,7β,7aα)-Hexahydro-2-(8-quinolinyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.28 LCMS/ 295.22 $[M + H]^+$	8
52		(3aα,4β,7β,7aα)-2-(2,3-Dihydro-1,4-benzodioxin-6-yl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.55 LCMS/ 302.23 $[M + H]^+$	8
53		(3aα,4β,7β,7aα)-Hexahydro-2-[2-oxo-4-(trifluoromethyl)-2H-1-benzopyran-7-yl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.38 LCMS/ 412.17 $[M + CH_3OH + H]^+$	8
54		(3aα,4β,7β,7aα)-Hexahydro-2-(4-methyl-2-oxo-2H-1-benzopyran-7-yl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.74 LCMS/ 326.20 $[M + H]^+$	8
55		(3aα,4β,7β,7aα)-2-(2,5-Dimethoxy-4-nitrophenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.70 LCMS/ 349.23 $[M + H]^+$	8

TABLE 2-continued

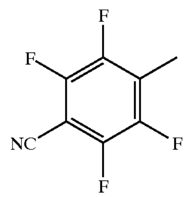
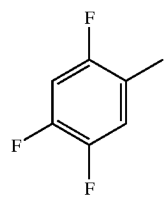
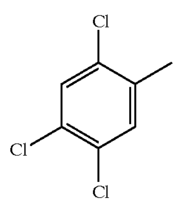
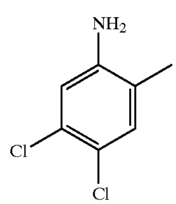
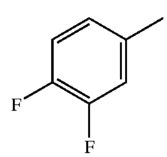
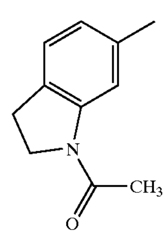
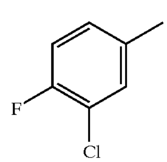
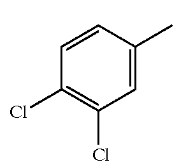
Ex. No.	G	Compound Name	Retention Time (Min.)/ Molecular Mass	Pro. of Ex.
56		(3aα,4β,7β,7aα)-2,3,5,6-Tetrafluoro-4-(octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)benzonitrile	2.97 LCMS	8
57		(3aα,4β,7β,7aα)-Hexahydro-2-(2,4,5-trifluorophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.90 LCMS	8
58		(3aα,4β,7β,7aα)-Hexahydro-2-(2,4,5-trichlorophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.64 LCMS/ 346.39 $[M]^+$	8
59		(3aα,4β,7β,7aα)-2-(2-Amino-4,5-dichlorophenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.23 LCMS	8
60		(3aα,4β,7β,7aα)-2-(3,4-Difluorophenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.91 LCMS/ 280.23 $[M + H]^+$	8
61		(3aα,4β,7β,7aα)-1-Acetyl-2,3-dihydro-6-(octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-1H-indole	2.43 LCMS/ 359.26 $[M + CH_3OH + H]^+$	8
62		(3aα,4β,7β,7aα)-2-(3-Chloro-4-fluorophenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.21 LCMS/ 328.14 $[M + CH_3OH + H]^+$	8
63		(3aα,4β,7β,7aα)-2-(3,4-Dichlorophenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.54 LCMS/ 311.79 $[M - H]^+$	8

TABLE 2-continued

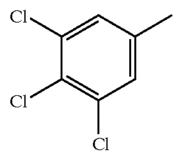
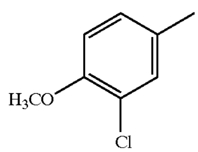
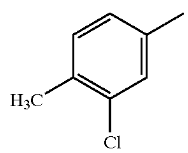
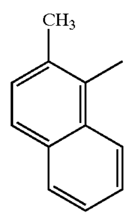
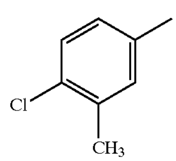
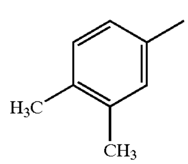
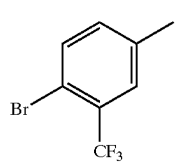
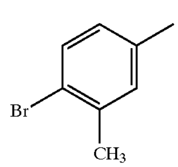
Ex. No.	G	Compound Name	Retention Time (Min.)/ Molecular Mass	Pro. of Ex.
64		(3aα,4β,7β,7aα)-Hexahydro-2-(3,4,5-trichlorophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	4.05 LCMS/ 378.10 $[M + CH_3OH + H]^+$	8
65		(3aα,4β,7β,7aα)-2-(3-Chloro-4-methoxyphenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.99 LCMS/ 308.11 $[M + H]^+$	8
66		(3aα,4β,7β,7aα)-2-(3-Chloro-4-methylphenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.39 LCMS/ 292.20 $[M + H]^+$	8
67		(3aα,4β,7β,7aα)-Hexahydro-2-(2-methyl-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.28 LCMS/ 308.23 $[M + H]^+$	8
68		(3aα,4β,7β,7aα)-2-(4-Chloro-3-methylphenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.40 LCMS/ 292.20 $[M + H]^+$	8
69		(3aα,4β,7β,7aα)-2-(3,4-Dimethylphenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.11 LCMS/ 272.23 $[M + H]^+$	8
70		(3aα,4β,7β,7aα)-2-[4-Bromo-3-(trifluoromethyl)phenyl]hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.76 LCMS/ 421.98 $[M + CH_3OH + H]^+$	8
71		(3aα,4β,7β,7aα)-2-(4-Bromo-3-methylphenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.50 LCMS/ 336.05 $[M + H]^+$	8

TABLE 2-continued

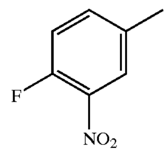
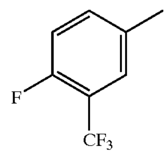
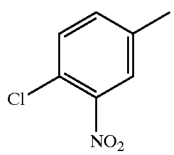
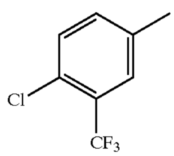
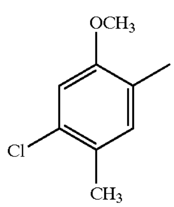
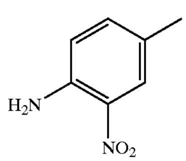
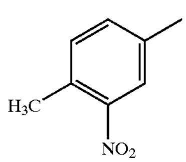
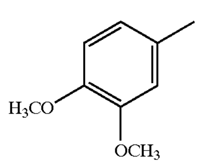
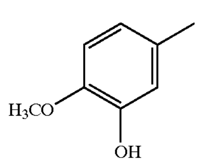
Ex. No.	G	Compound Name	Retention Time (Min.)/ Molecular Mass	Pro. of Ex.
72		(3aα,4β,7β,7aα)-2-(4-Fluoro-3-nitrophenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.80 LCMS/ 305.25 $[M - H]^+$	8
73		(3aα,4β,7β,7aα)-2-[4-Fluoro-3-(trifluoromethyl)phenyl]hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.45 LCMS/ 362.26 $[M + CH_3OH + H]^+$	8
74		(3aα,4β,7β,7aα)-2-(4-Chloro-3-nitrophenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.19 LCMS/ 322.86 $[M]^+$	8
75		(3aα,4β,7β,7aα)-2-[4-Chloro-3-(trifluoromethyl)phenyl]hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.68 LCMS/ 345.83 $[M]^+$	8
76		(3aα,4β,7β,7aα)-2-(4-Chloro-2-methoxy-5-methylphenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.31 LCMS/ 322.20 $[M + H]^+$	8
77		(3aα,4β,7β,7aα)-2-(4-Amino-3-nitrophenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.34 LCMS/ 302.27 $[M - H]^-$	8
78		(3aα,4β,7β,7aα)-Hexahydro-2-(4-methyl-3-nitrophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.02 LCMS/ 335.20 $[M + CH_3OH + H]^+$	8
79		(3aα,4β,7β,7aα)-2-(3,4-Dimethoxyphenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.35 LCMS/ 304.25 $[M + H]^+$	8
80		(3aα,4β,7β,7aα)-Hexahydro-2-(3-hydroxy-4-methoxyphenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	0.98 LCMS/ 321.19 $[M + CH_3OH]^+$	8

TABLE 2-continued

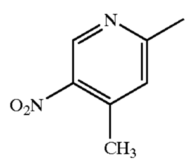
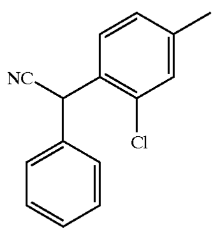
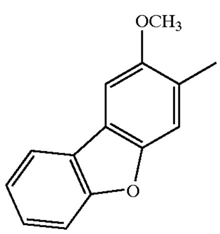
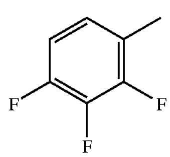
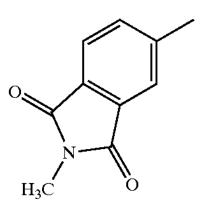
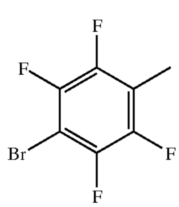
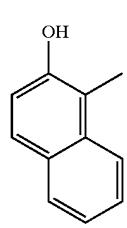
Ex. No.	G	Compound Name	Retention Time (Min.)/ Molecular Mass	Pro. of Ex.
81		(3aα,4β,7β,7aα)-Hexahydro-2-(4-methyl-5-nitro-2-pyridinyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	0.54 LCMS/ 304.20 $[M + H]^+$	8
82		(3aα,4β,7β,7aα)-2-Chloro-4-(octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-α-phenylbenzeneacetonitrile	3.67 LCMS/ 423.8 $[M + CH_3OH]^+$	8
83		(3aα,4β,7β,7aα)-Hexahydro-2-(2-methoxy-3-dibenzofuranyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.66 LCMS/ 364.25 $[M + H]^+$	8
84		(3aα,4β,7β,7aα)-Hexahydro-2-(2,3,4-trifluorophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.06 LCMS/ 298.14 $[M + H]^+$	8
85		(3aα,4β,7β,7aα)-2-(2,3-Dihydro-2-methyl-1,3-dioxo-1H-isoindol-5-yl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.70 LCMS/ 359.22 $[M + CH_3OH + H]^+$	8
86		(3aα,4β,7β,7aα)-2-(4-Bromo-2,3,5,6-tetrafluorophenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.72 LCMS/ 426.07 $[M + CH_3OH + H]^+$	8
87		(3aα,4β,7β,7aα)-Hexahydro-2-(2-hydroxy-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.52 LCMS/ 308.26 $[M - H]^-$	8

TABLE 2-continued

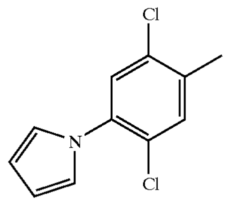
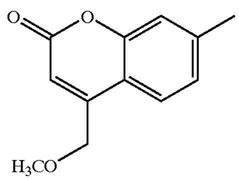
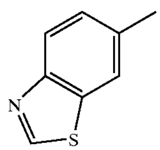
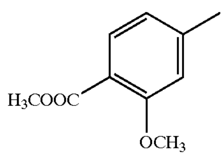
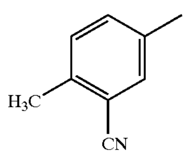
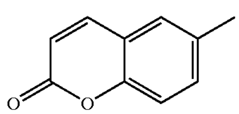
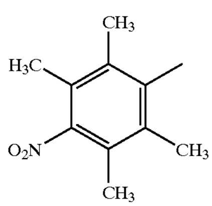
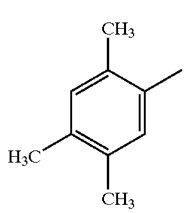
Ex. No.	G	Compound Name	Retention Time (Min.)/ Molecular Mass	Pro. of Ex.
88		(3aα,4β,7β,7aα)-2-[2,5-Dichloro-4-(1H-pyrrol-1-yl)phenyl]hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.70 LCMS/ 376.64 $[M-H]^+$	8
89		(3aα,4β,7β,7aα)-Hexahydro-2-[4-(methoxymethyl)-2-oxo-2H-1-benzopyran-7-yl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.79 LCMS/ 356.26 $[M+H]^+$	8
90		(3aα,4β,7β,7aα)-2-(6-Benzothiazolyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.46 LCMS/ 301.19 $[M+H]^+$	8
91		(3aα,4β,7β,7aα)-2-Methoxy-4-(octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)benzoic acid methyl ester	2.75 LCMS/ 332.25 $[M+H]^+$	8
92		(3aα,4β,7β,7aα)-2-Methyl-5-(octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)benzonitrile	2.80 LCMS/ 315.26 $[M+CH_3OH+H]^+$	8
93		(3aα,4β,7β,7aα)-Hexahydro-2-(2-oxo-2H-1-benzopyran-6-yl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.45 LCMS/ 312.20 $[M+H]^+$	8
94		(3aα,4β,7β,7aα)-Hexahydro-2-(2,3,5,6-tetramethyl-4-nitrophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.59 LCMS/ 377.25 $[M+CH_3OH+H]^+$	8
95		(3aα,4β,7β,7aα)-Hexahydro-2-(2,4,5-trimethylphenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.33 LCMS/ 286.30 $[M+H]^+$	8

TABLE 2-continued

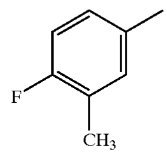
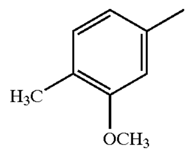
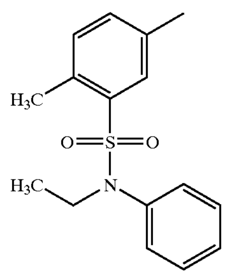
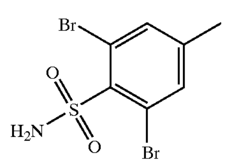
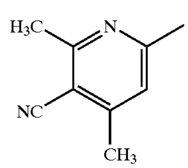
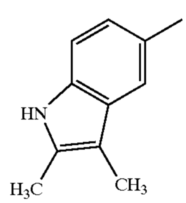
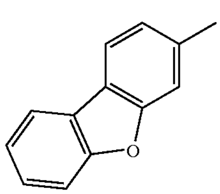
Ex. No.	G	Compound Name	Retention Time (Min.)/ Molecular Mass	Pro. of Ex.
96		(3aα,4β,7β,7aα)-2-(4-Fluoro-3-methylphenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.00 LCMS/ 276.23 $[M + H]^+$	8
97		(3aα,4β,7β,7aα)-Hexahydro-2-(3-methoxy-4-methylphenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.05 LCMS/ 288.23 $[M + H]^+$	8
98		(3aα,4β,7β,7aα)-N-Ethyl-2-methyl-5-(octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-N-phenylbenzenesulfonamide	3.56 LCMS/ 441.26 $[M + H]^+$	8
99		(3aα,4β,7β,7aα)-2,6-Dibromo-4-(octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)benzenesulfonamide	2.25 LCMS	8
100		(3aα,4β,7β,7aα)-2,4-Dimethyl-6-(octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-3-pyridinecarbonitrile	2.75 LCMS/ 298.23 $[M + H]^+$	8
101		(3aα,4β,7β,7aα)-2-(2,3-Dimethyl-1H-indol-5-yl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.00 LCMS/ 311.26 $[M + H]^+$	8
102		(3aα,4β,7β,7aα)-2-(3-Dibenzofuranyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.72 LCMS/ 366.23 $[M + CH_3OH + H]^+$	8

TABLE 2-continued

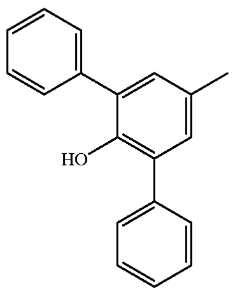
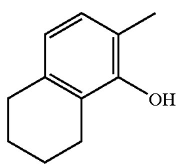
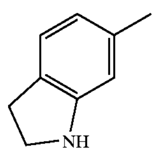
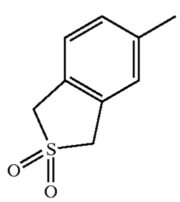
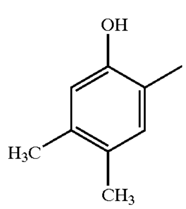
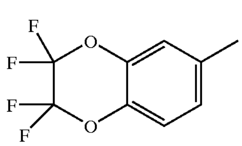
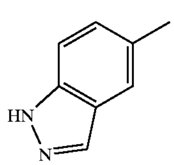
Ex. No.	G	Compound Name	Retention Time (Min.)/ Molecular Mass	Pro. of Ex.
103		(3aα,4β,7β,7aα)-Hexahydro-2-(2'-hydroxy[1,1':3',1"-terphenyl]-5'-yl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.70 LCMS/ 412.23 $[M + H]^+$	8
104		(3aα,4β,7β,7aα)-Hexahydro-2-(5,6,7,8-tetrahydro-3-hydroxy-2-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.24 LCMS/ 312.32 $[M + H]^+$	8
105		(3aα,4β,7β,7aα)-2-(2,3-Dihydro-1H-indol-6-yl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.42 LCMS/ 285.29 $[M + H]^+$	8
106		(3aα,4β,7β,7aα)-2-(1,3-Dihydro-2,2-dioxidobenzo[c]thiophen-5-yl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	1.99 LCMS/ 366.26 $[M + CH_3OH + H]^+$	8
107		(3aα,4β,7β,7aα)-Hexahydro-2-(2-hydroxy-4,5-dimethylphenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.78 LCMS/ 286.32 $[M - H]^+$	8
108		(3aα,4β,7β,7aα)-2-(2,3-Dihydro-2,2,3,3-tetrafluoro-1,4-benzodioxin-6-yl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.82 LCMS/ 406.19 $[M + CH_3OH + H]^+$	8
109		(3aα,4β,7β,7aα)-Hexahydro-2-(1H-indazol-5-yl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.13 LCMS/ 284.23 $[M + H]^+$	8

TABLE 2-continued

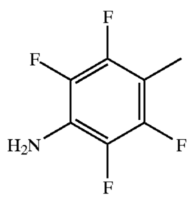
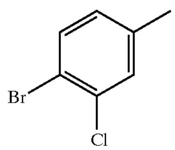
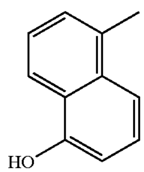
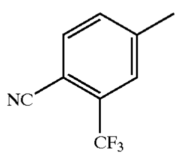
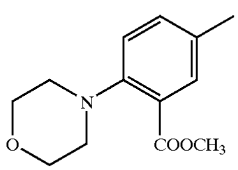
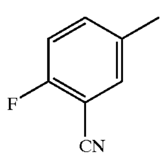
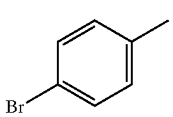
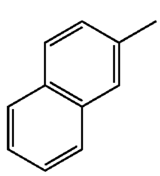
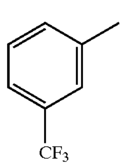
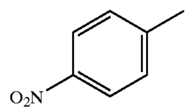
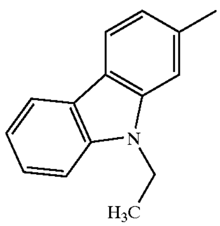
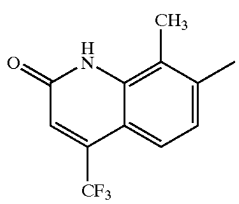
Ex. No.	G	Compound Name	Retention Time (Min.)/ Molecular Mass	Pro. of Ex.
110		(3aα,4β,7β,7aα)-2-(4-Amino-2,3,5,6-tetrafluorophenyl)-hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.60 LCMS/ 363.22 $[M + CH_3OH + H]^+$	8
111		(3aα,4β,7β,7aα)-2-(4-Bromo-3-chlorophenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.64 LCMS/ 389.64 $[M + CH_3OH + H]^+$	8
112		(3aα,4β,7β,7aα)-Hexahydro-2-(5-hydroxy-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.48 LCMS/ 308.27 $[M - H]^-$	8
113		(3aα,4β,7β,7aα)-4-(Octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile	3.28 LCMS/ 337.16 $[M + H]^+$	8
114		(3aα,4β,7β,7aα)-2-(4-Morpholinyl)-5-(octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)benzoic acid methyl ester	2.72 LCMS/ 387.17 $[M + H]^+$	8
115		(3aα,4β,7β,7aα)-2-Fluoro-5-(octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)benzonitrile	2.69 LCMS/ 319.26 $[M + CH_3OH + H]^+$	8
116		(3aα,4β,7β,7aα)-2-(4-Bromophenyl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	5.80 LCMS/ 393.0 $[M + H]^+$	8
117		(3aα,4β,7β,7aα)-Hexahydro-2-(2-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	6.92 LCMS/ 333.7 $[M + H]^+$	8
118		(3aα,4β,7β,7aα)-Hexahydro-2-[3-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.27 LCMS/ 312.2 $[M + H]^+$	8

TABLE 2-continued

Ex. No.	G	Compound Name	Retention Time (Min.)/ Molecular Mass	Pro. of Ex.
119		(3aα,4β,7β,7aα)-Hexahydro-2-(4-nitrophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.88 LCMS/ 343.2 $[M + H]^+$	8
120		(3aα,4β,7β,7aα)-2-(9-Ethyl-9H-carbazol-3-yl)hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.73 LCMS/ 360.1 $[M + H]^+$	8
121		(3aα,4β,7β,7aα)-2-[1,2-Dihydro-8-methyl-2-oxo-4-(trifluoromethyl)-7-quinolinyl]hexahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.11 LCMS/ 393.0 $[M + H]^+$	8

EXAMPLES 122 TO 164

Further compounds of the present invention were prepared by procedures analogous to those described above. Table 3 provides the compound name and structure, retention time, as well as the Example number of the procedure on which the preparation of Table 3 was based, for the compounds of Examples 122 to 164. The chromatography techniques used to determine the compound retention times of Table 3 are as follows:

LCMS=YMC S5 ODS column, 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 4 minutes containing 0.1% TFA; 4 mL/min, monitoring at 220 nm.

LC=YMC S5 ODS column 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm.

TABLE 3

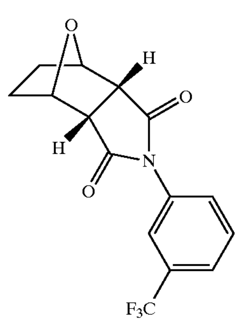
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
122		(3aα,4α,7α,7aα)-Hexahydro-2-[3-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.66 LCMS	27

TABLE 3-continued

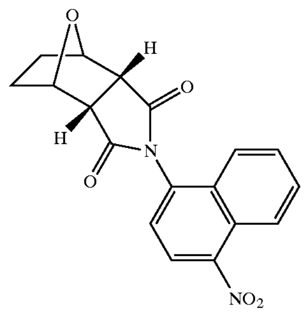
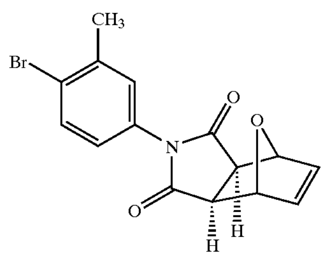
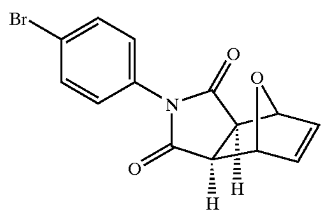
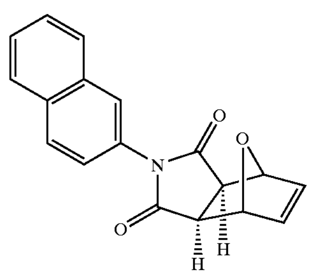
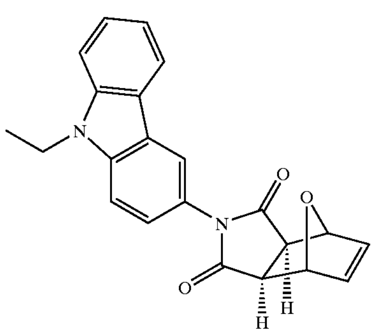
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
123		(3aα,4α,7α,7aα)-Hexahydro-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.76 LCMS	27
124		(3aα,4β,7β,7aα)-2-(4-Bromo-3-methylphenyl)-3a,4,7,7a-tetrahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	6.36 LCMS	8
125		(3aα,4β,7β,7aα)-2-(4-Bromophenyl)-3a,4,7,7a-tetrahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	5.72 LCMS	8
126		(3aα,4β,7β,7aα)-3a,4,7,7a-Tetrahydro-2-(2-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	5.92 LCMS	8
127		(3aα,4β,7β,7aα)-2-(9-Ethyl-9H-carbazol-3-yl)-3a,4,7,7a-tetrahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.73 LCMS	8

TABLE 3-continued

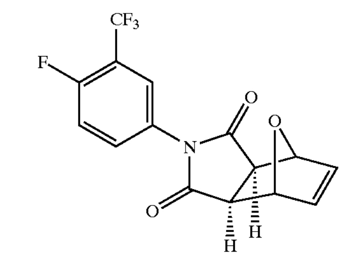
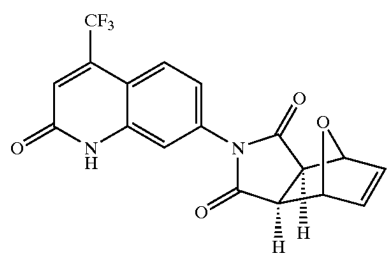
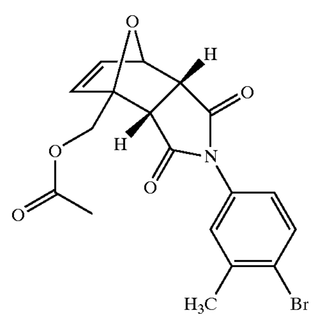
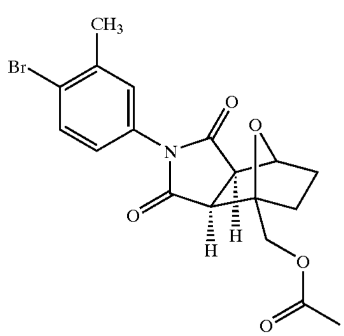
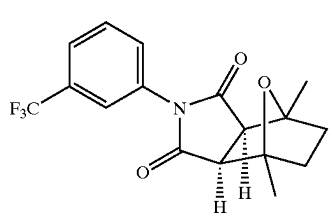
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
128		(3aα,4β,7β,7aα)-2-[4-Fluoro-3-(trifluoromethyl)phenyl]-3a,4,7,7a-tetrahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.40 LCMS	8
129		(3aα,4β,7β,7aα)-2-[1,2-Dihydro-8-methyl-2-oxo-4-(trifluoromethyl)-7-quinolinyl]-3a,4,7,7a-tetrahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.14 LCMS	8
130		(3aα,4α,7α,7aα)-4-[(Acetyloxy)methyl]-2-(4-bromo-3-methylphenyl)-3a,4,7,7a-tetrahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.95 LC	4
131		(3aα,4β,7β,7aα)-4-[(Acetyloxy)methyl]-2-(4-bromo-3-methylphenyl)-3a,4,7,7a-tetrahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.97 LCMS	5
132		(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-[3-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.08 LC	20

TABLE 3-continued

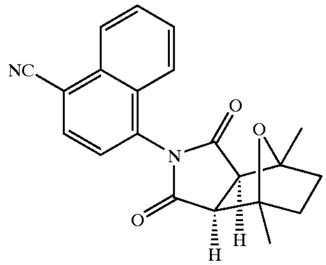
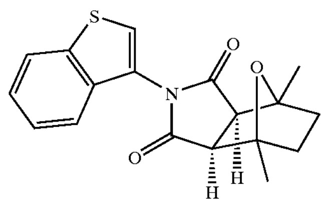
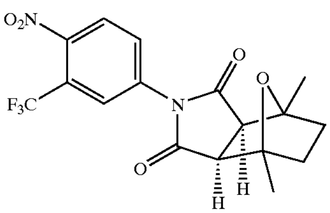
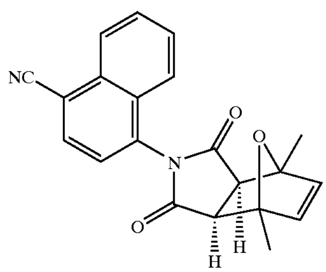
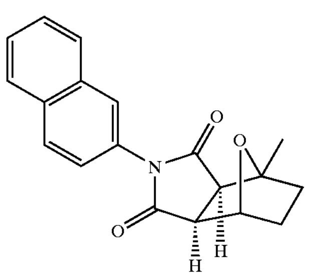
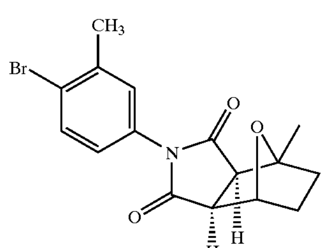
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
133		(3aα,4β,7β,7aα)-4-(Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-1-naphthalenecarbonitrile	3.00 LC	20
134		(3aα,4β,7β,7aα)-(Benzo[b]thiophen-3-yl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.61 LC	20
135		(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-[4-nitro-3-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.21 LC	20
136		(3aα,4β,7β,7aα)-4-(1,3,3a,4,7,7a-Hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-1-naphthalenecarbonitrile	2.94 LC	32
137		(3aα,4α,7α,7aα)-Hexahydro-4-methyl-2-(2-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.88 LC	3
138		(3aα,4β,7β,7aα)-2-(4-Bromo-3-methylphenyl)hexahydro-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.11 LC	3

TABLE 3-continued

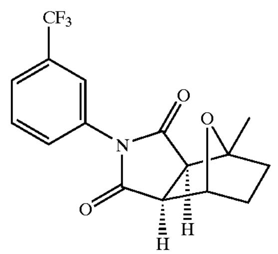
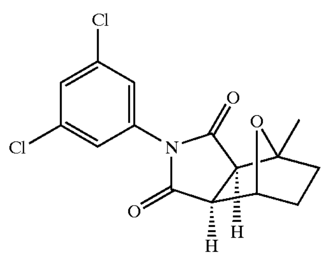
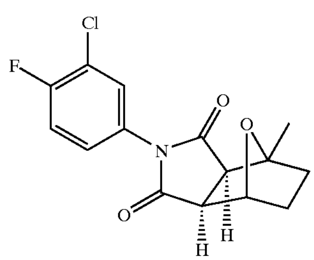
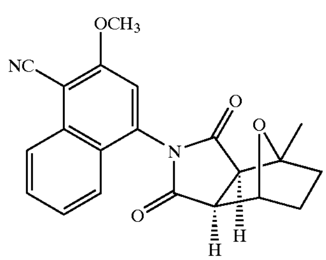
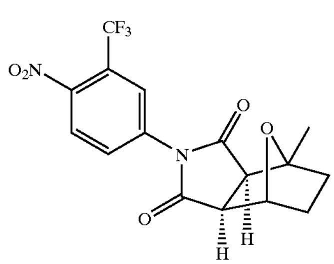
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
139		(3aα,4β,7β,7aα)-Hexahydro-4-methyl-2-[3-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.90 LC	3
140		(3aα,4β,7β,7aα)-2-(3,5-Dichlorophenyl)hexahydro-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.31 LC	3
141		(3aα,4β,7β,7aα)-2-(3-Chloro-4-fluorophenyl)-hexahydro-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.72 LC	3
142		(3aα,4β,7β,7aα)-2-Methoxy-4-(octahydro-1,3-dioxo-4-methyl-4,7-epoxy-2H-isoindol-2-yl)-1-naphthalenecarbonitrile	2.72 LC	3
143		(3aα,4β,7β,7aα)-Hexahydro-4-methyl-2-[4-nitro-3-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.10 LC	3

TABLE 3-continued

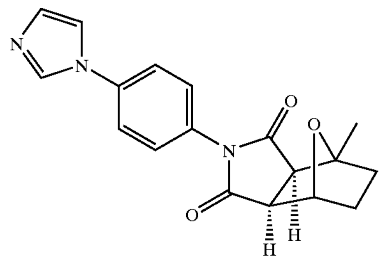
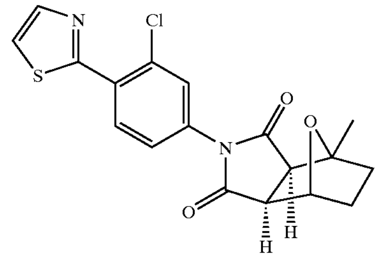
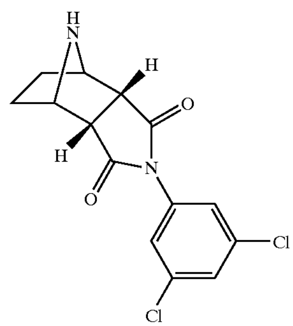
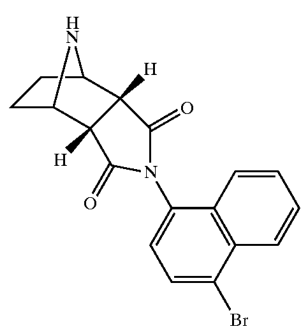
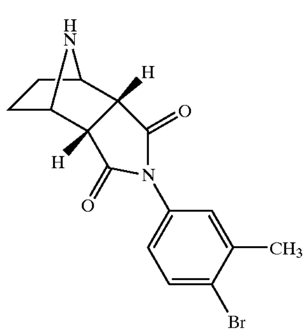
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
144		(3aα,4β,7β,7aα)-Hexahydro-2-[4-(1H-imidazol-1-yl)phenyl]-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	1.16 LC	3
145		(3aα,4β,7β,7aα)-2-[3-Chloro-4-(2-thiazolyl)phenyl]hexahydro-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.81 LC	3
146		(3aα,4α,7α,7aα)-2-(3,5-Dichlorophenyl)hexahydro-4,7-imino-1H-isoindole-1,3(2H)-dione	2.72 LC	31
147		(3aα,4α,7α,7aα)-2-(4-Bromo-1-naphthalenyl)hexahydro-4,7-imino-1H-isoindole-1,3(2H)-dione	2.95 LC	31
148		(3aα,4α,7α,7aα)-2-(4-Bromo-3-methylphenyl)hexahydro-4,7-imino-1H-isoindole-1,3(2H)-dione	2.65 LC	31

TABLE 3-continued

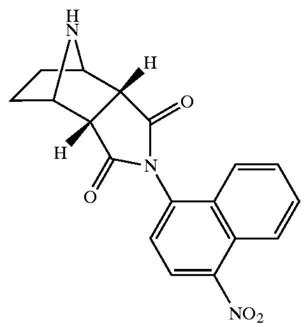
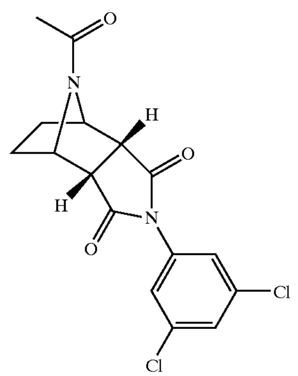
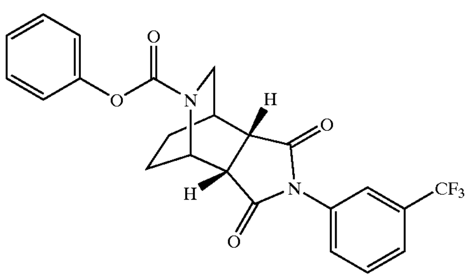
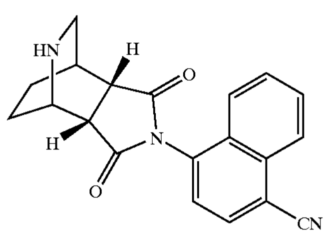
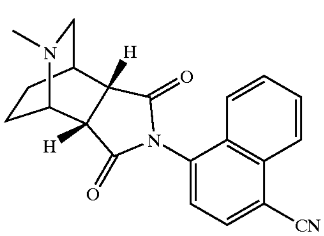
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
149		(3aα,4α,7α,7aα)-Hexahydro-2-(4-nitro-1-naphthalenyl)-4,7-imino-1H-isoindole-1,3(2H)-dione	2.49 LC	31
150		(3aα,4α,7α,7aα)-8-Acetyl-2-(3,5-dichlorophenyl)hexahydro-4,7-imino-1H-isoindole-1,3(2H)-dione	3.53 LC	31, 12
151		(3aα,4α,7α,7aα)-Octahydro-1,3-dioxo-2-[3-(trifluoromethyl)phenyl]-4,7-ethano-5H-pyrrolo[3,4-c]pyridine-5-carboxylic acid phenyl ester	3.397 LC	9
152		(3aα,4α,7α,7aα)-4-(Octahydro-1,3-dioxo-4,7-ethano-2H-pyrrolo[3,4-c]pyridin-2-yl)-1-naphthalenecarbonitrile	1.74 LC	11
153		(3aα,4α,7α,7aα)-4-(Octahydro-5-methy)-1,3-dioxo-4,7-ethano-2H-pyrrolo[3,4-c]pyridin-2-yl)-1-naphthalenecarbonitrile	1.71 LC	14

TABLE 3-continued

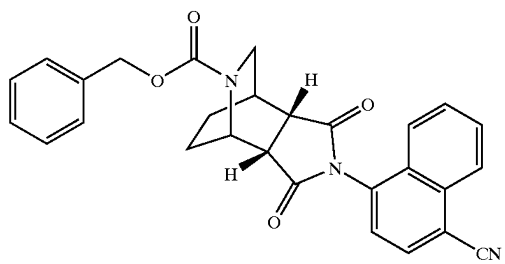
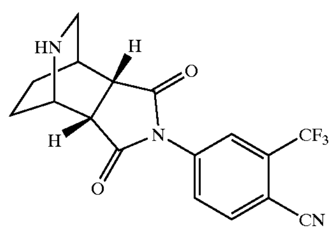
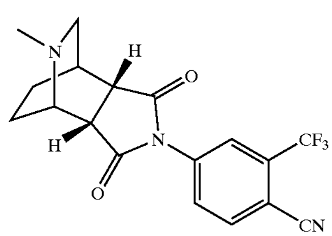
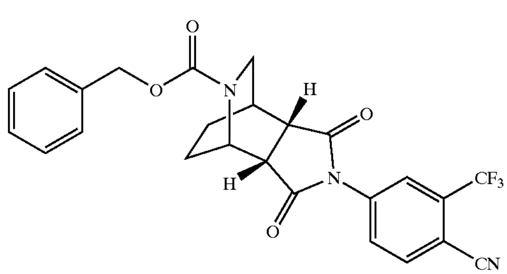
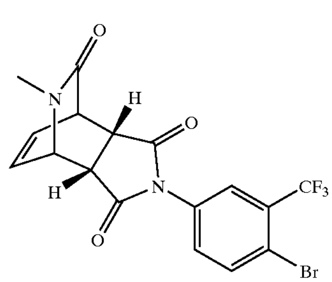
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
154		(3aα,4α,7α,7aα)-2-(4-Cyano-1-naphthalenyl)octahydro-1,3-dioxo-4,7-etheno-5H-pyrrolo[3,4-c]pyridine-5-carboxylic acid phenylmethyl ester	3.40 LC	10
155		(3aα,4α,7α,7aα)-4-(Octahydro-1,3-dioxo-4,7-ethano-2H-pyrrolo[3,4-c]pyridin-2-yl)-2-(trifluoromethyl)benzonitrile	1.74 LC	11
156		(3aα,4α,7α,7aα)-4-(Octahydro-5-methyl-1,3-dioxo-4,7-ethano-2H-pyrrolo[3,4-c]pyridin-2-yl)-2-(trifluoromethyl)benzonitrile	1.65 LC	14
157		(3aα,4α,7α,7aα)-2-[4-Cyano-3-(trifluoromethyl)phenyl]octa-hydro-1,3-dioxo-4,7-etheno-5H-pyrrolo[3,4-c]pyridine-5-carboxylic acid phenylmethyl ester	3.53 LC	10
158		(3aα,4α,7α,7aα)-2-[4-Bromo-3-(trifluoromethyl)phenyl]tetra-hydro-5-methyl-4,7-etheno-1H-pyrrolo[3,4-c]pyridine-1,3,6(2H,5H)-trione	2.95 LCMS	34

TABLE 3-continued

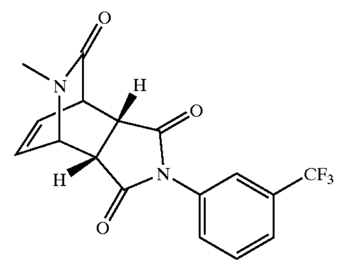
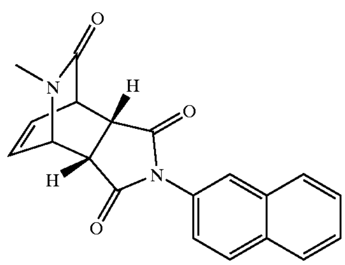
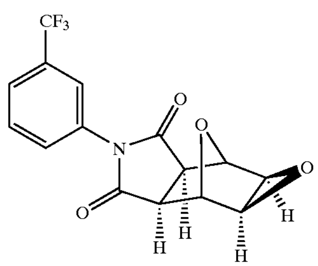
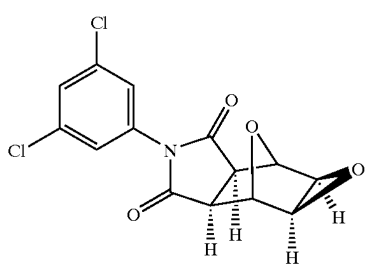
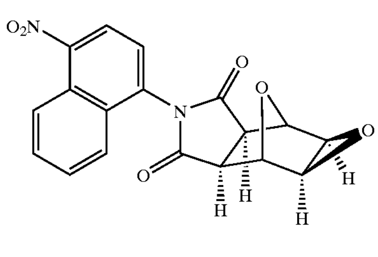
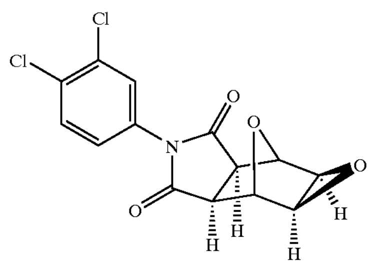
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
159		(3aα,4α,7α,7aα)-Tetrahydro-5-methyl-2-[3-(trifluoromethyl)phenyl]-4,7-etheno-1H-pyrrolo[3,4-c]pyridine-1,3,6(2H,5H)-trione	2.53 LCMS	34
160		(3aα,4α,7α,7aα)-Tetrahydro-5-methyl-2-(2-naphthalenyl)-4,7-etheno-1H-pyrrolo[3,4-c]pyridine-1,3,6(2H,5H)-trione	2.58 LCMS	34
161		(1aα,2β,2aα,5aα,6β,6aα)-Hexahydro-4-[3-(trifluoromethyl)phenyl]-2,6-epoxy-3H-oxireno[f]iso-indole-3,5(4H)-dione	1.80 LCMS	28
162		(1aα,2β,2aα,5aα,6β,6aα)-4-(3,5-Dichlorophenyl)-hexahydro-2,6-epoxy-3H-oxireno[f]isoindole-3,5(4H)-dione	1.45 LCMS	28
163		(1aα,2β,2aα,5aα,6β,6aα)-Hexahydro-4-(4-nitro-1-naphthalenyl)-2,6-epoxy-3H-oxireno[f]isoindole-3,5(4H)-dione	1.52 LCMS	28

TABLE 3-continued

Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
164		(1aα,2β,2aα,5aα,6β,6aα)-4-(3,4-Dichlorophenyl)-hexahydro-2,6-epoxy-3H-oxireno[f]isoindole-3,5(4H)-dione	3.21 LCMS	28

EXAMPLES 165 TO 203

Additional compounds of the present invention were prepared and are described further below in Table 4. Table 4 sets forth the compound name and structure, as well as the Example number of the procedure on which the preparation of Table 4 was based, for the compounds of Examples 165 to 203.

TABLE 4

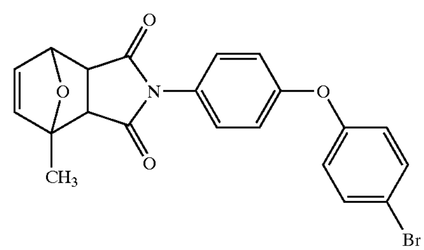
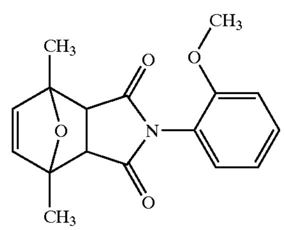
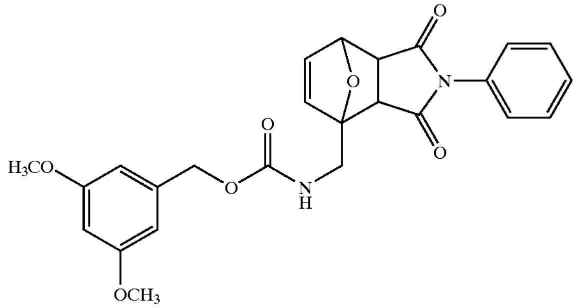
Ex. No.	Compound Structure	Compound Name	Pro. of Ex.
165		2-[4-(4-Bromo-phenoxy)phenyl]-3a,4,7,7a-tetrahydro-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	32
166		3a,4,7,7a-Tetrahydro-2-(2-methoxyphenyl)-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	32
167		[(1,2,3,3a,7,7a-Hexahydro-2-phenyl-4,7-epoxy-4H-isoindol-4-yl)methyl]carbamic acid (3,5-dimethoxyphenyl)methyl ester	21–26

TABLE 4-continued

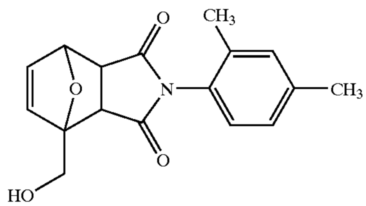
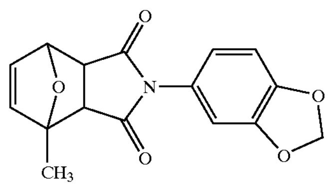
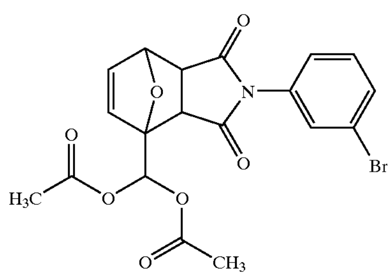
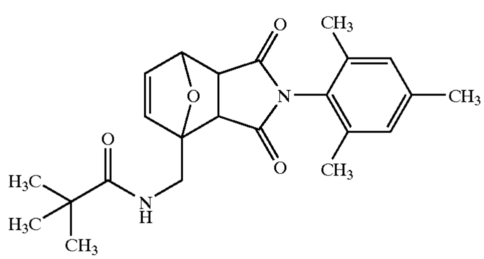
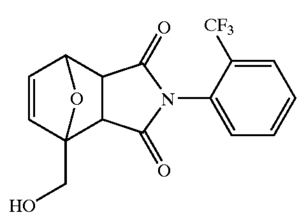
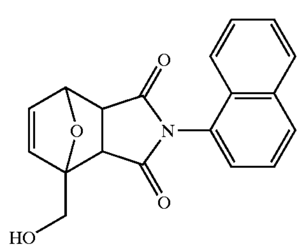
Ex. No.	Compound Structure	Compound Name	Pro. of Ex.
168		2-(2,4-Dimethylphenyl)-3a,4,7,7a-tetrahydro-4-(hydroxymethyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	21–26
169		2-(1,3-Benzodioxol-5-yl)-3a,4,7,7a-tetrahydro-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	32
170		4-[Bis(acetyloxy)methyl]-2-(3-bromophenyl)-3a,4,7,7a-tetrahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	21–26
171		N-[[1,2,3,3a,7,7a-Hexahydro-2-(2,4,6-trimethylphenyl)-4,7-epoxy-4H-isoindol-4-yl]methyl]-2,2-dimethylpropanamide	21–26
172		3a,4,7,7a-Tetrahydro-4-(hydroxymethyl)-2-[2-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione	21–26
173		3a,4,7,7a-Tetrahydro-4-(hydroxymethyl)-2-(1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	21–26

TABLE 4-continued

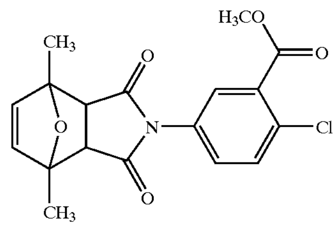
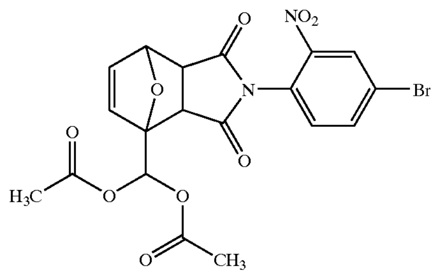
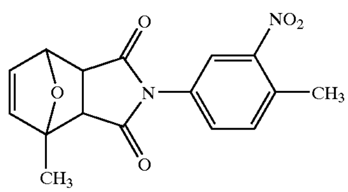
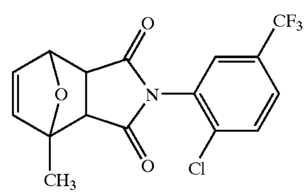
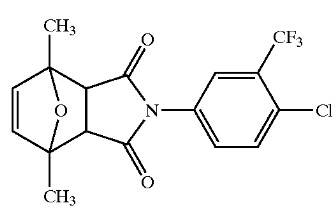
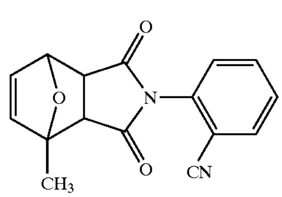
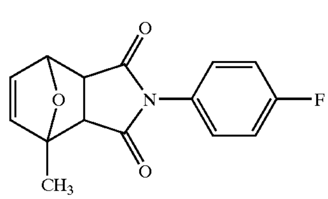
Ex. No.	Compound Structure	Compound Name	Pro. of Ex.
174		2-Chloro-5-(1,3,3a,4,7,7a-hexahydro-4,7-dimethyl-4,7-epoxy-2H-isoindol-2-yl)benzoic acid methyl ester	32
175		4-[Bis(acetyloxy)methyl]-2-(4-bromo-2-nitrophenyl)-3a,4,7,7a-tetrahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione	21–26
176		3a,4,7,7a-Tetrahydro-4-methyl-2-(4-methyl-3-nitrophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	32
177		2-[2-Chloro-5-(trifluoromethyl)phenyl]-3a,4,7,7a-tetrahydro-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	32
178		2-[4-Chloro-3-(trifluoromethyl)phenyl]-3a,4,7,7a-tetrahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	32
179		2-(1,3,3a,4,7,7a-Hexahydro-4-methyl-4,7-epoxy-2H-isoindol-2-yl)benzonitrile	32
180		2-(4-Fluorophenyl)-3a,4,7,7a-tetrahydro-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	32

TABLE 4-continued

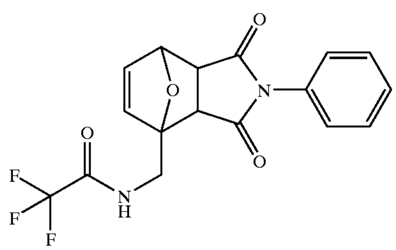
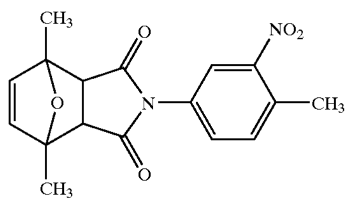
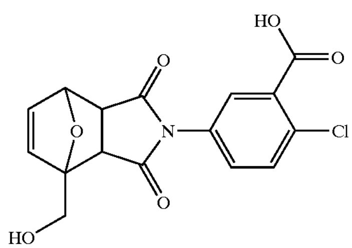
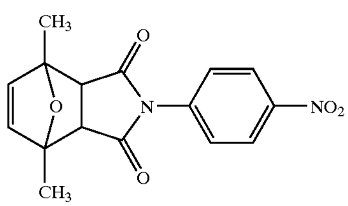
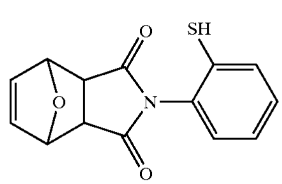
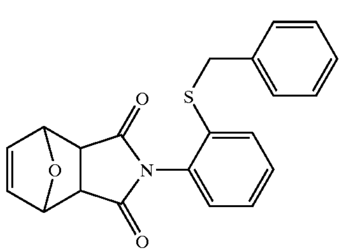
Ex. No.	Compound Structure	Compound Name	Pro. of Ex.
181		2,2,2-Trifluoro-N-[(1,2,3,3a,7,7a-hexahydro-2-phenyl-4,7-epoxy-4H-isoindol-4-yl)methyl]acetamide	21–26
182		3a,4,7,7a-Tetrahydro-4,7-dimethyl-2-(4-methyl-3-nitrophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	32
183		2-Chloro-5-[1,3,3a,4,7,7a-hexahydro-4-(hydroxymethyl)-4,7-epoxy-2H-isoindol-2-yl]benzoic acid	21–26
184		3a,4,7,7a-Tetrahydro-4,7-dimethyl-2-(4-nitrophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	32
185		3a,4,7,7a-Tetrahydro-2-(2-mercaptophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione	32
186		3a,4,7,7a-Tetrahydro-2-[2-[(phenylmethyl)thio]-phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione	32

TABLE 4-continued

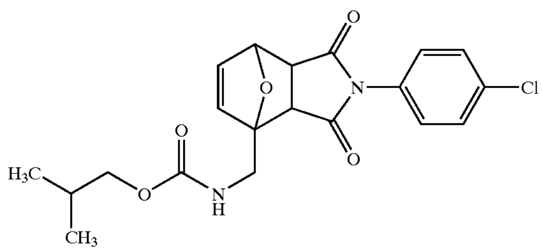
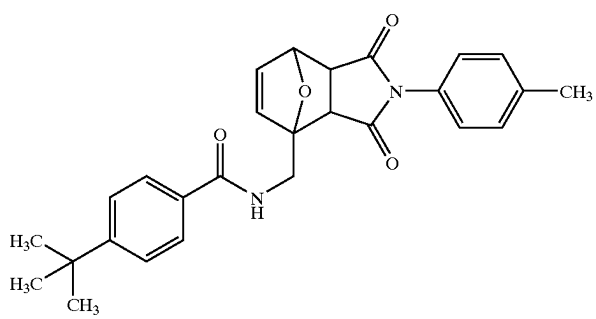
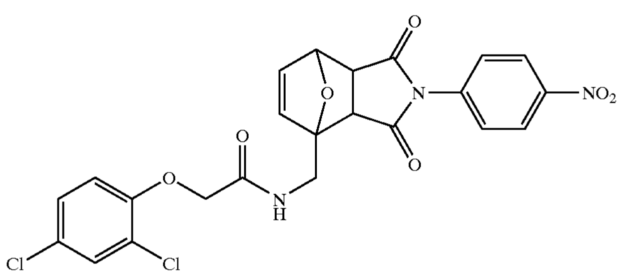
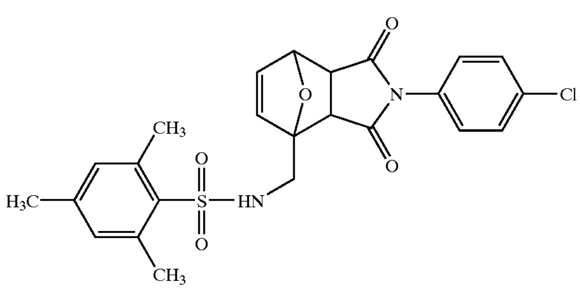
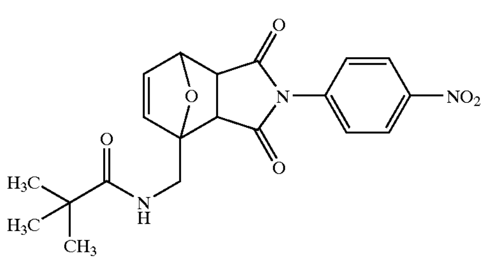
Ex. No.	Compound Structure	Compound Name	Pro. of Ex.
187		[[2-(4-Chlorophenyl)-1,2,3,3a,7,7a-hexahydro-4,7-epoxy-4H-isoindol-4-yl]methyl]carbamic acid 2-methylpropyl ester	21–26
188		4-(1,1-Dimethylethyl)-N-[[1,2,3,3a,7,7a-hexahydro-2-(4-methylphenyl)-4,7-epoxy-4H-isoindol-4-yl]methyl]benzamide	21–26
189		2,4-Dichloro-N-[[1,2,3,3a,7,7a-hexahydro-2-(4-nitrophenyl)-4,7-epoxy-4H-isoindol-4-yl]methyl]benzamide	21–26
190		N-[[2-(4-Chlorophenyl)-1,2,3,3a,7,7a-hexahydro-4,7-epoxy-4H-isoindol-4-yl]methyl]-2,4,6-trimethylbenzenesulfonamide	21–26
191		N-[[1,2,3,3a,7,7a-Hexahydro-2-(4-nitrophenyl)-4,7-epoxy-4H-isoindol-4-yl]methyl]-2,2-dimethylpropanamide	21–26

TABLE 4-continued

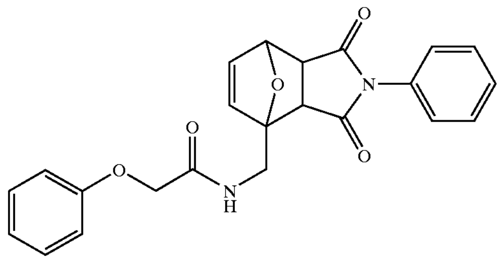
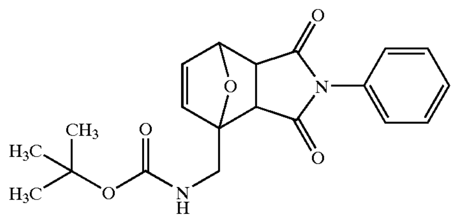
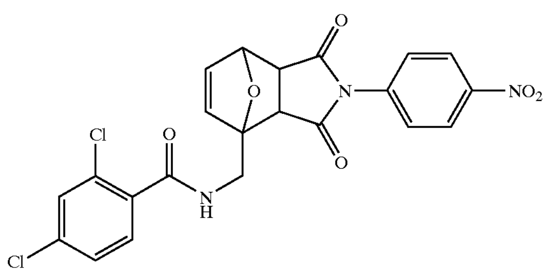
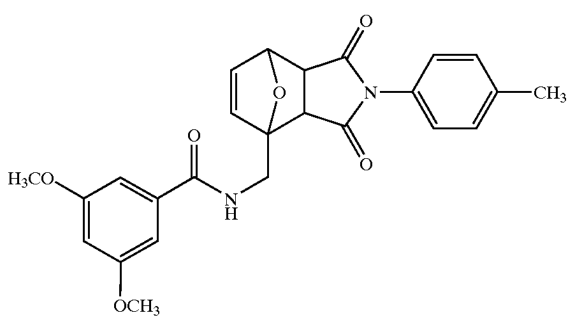
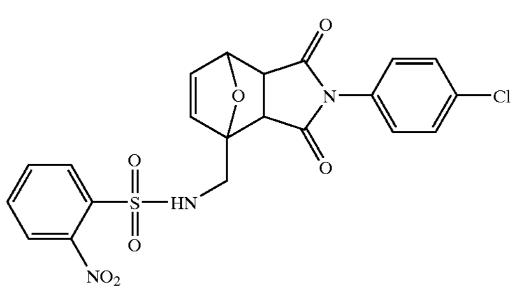
Ex. No.	Compound Structure	Compound Name	Pro. of Ex.
192		N-[(1,2,3,3a,7,7a-Hexahydro-2-phenyl-4,7-epoxy-4H-isoindol-4-yl)methyl]-2-phenoxyacetamide	21–26
193		[(1,2,3,3a,7,7a-Hexahydro-2-phenyl-4,7-epoxy-4H-isoindol-4-yl)methyl]carbamic acid 1,1-dimethylethyl ester	21–26
194		2-(2,4-Dichlorophenoxy)-N-[[1,2,3,3a,7,7a-hexahydro-2-(4-nitrophenyl)-4,7-epoxy-4H-isoindol-4-yl]methyl]acetamide	21–26
195		N-[[1,2,3,3a,7,7a-Hexahydro-2-(4-methylphenyl)-4,7-epoxy-4H-isoindol-4-yl]methyl]-3,5-dimethoxybenzamide	21–26
196		N-[[2-(4-Chlorophenyl)-1,2,3,3a,7,7a-hexahydro-4,7-epoxy-4H-isoindol-4-yl]methyl]-2-nitrobenzenesulfonamide	21–26

TABLE 4-continued

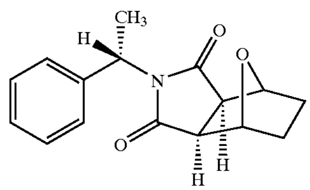
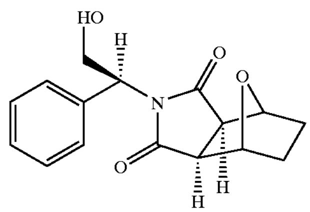
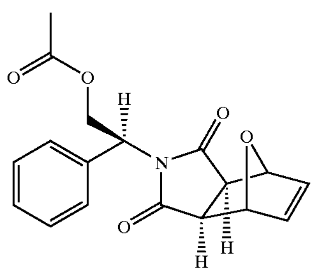
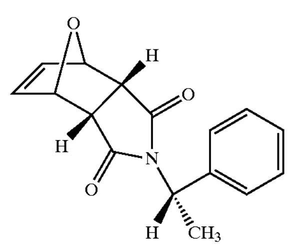
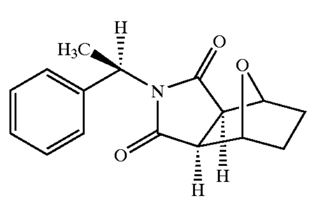
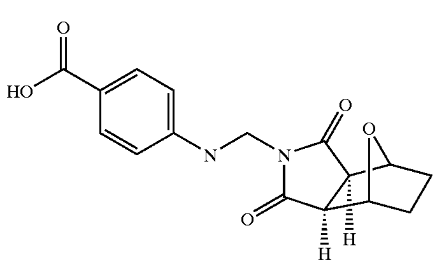
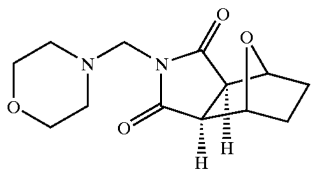
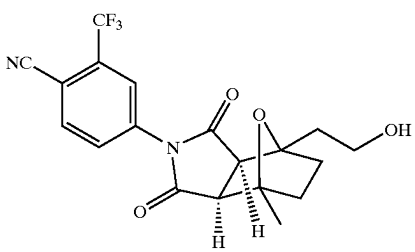
Ex. No.	Compound Structure	Compound Name	Pro. of Ex.
197		(3aα,4β,7β,7aα)-Hexahydro-2-[(1S)-1-phenylethyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	8
198		(3aα,4β,7β,7aα)-Hexahydro-2-[(1S)-2-hydroxy-1-phenylethyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	8
199		(3aα,4β,7β,7aα)-2-[(1S)-2-(Acetyloxy)-1-phenylethyl]-3a,4,7,7a-tetrahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	8
200		(3aα,4α,7α,7aα)-3a,4,7,7a-Tetrahydro-2-[(1S)-1-phenylethyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	8
201		(3aα,4β,7β,7aα)-Hexahydro-2-[(1R)-1-phenylethyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	8
202		(3aα,4β,7β,7aα)-4-[[[(Octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)methyl]amino]benzoic acid.	8

TABLE 4-continued

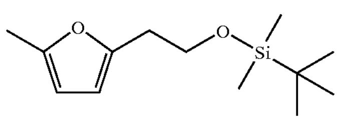
Ex. No.	Compound Structure	Compound Name	Pro. of Ex.
203		(3aα,4β,7β,7aα)-Hexahydro-2-(4-morpholinylmethyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	8

EXAMPLE 204

(3aα,4β,7β,7aα)-4-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (204D/25B)

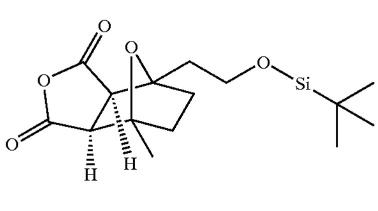


A. 2-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-5-methylfuran (204A)



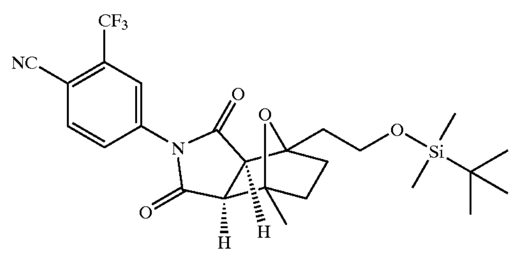
To a solution of compound 21A (2.00 g, 15.9 mmol) in DMF (50 mL) was added imidazole (1.62 g, 23.9 mmol), followed by tert-butyldimethylsilyl chloride (2.63 g, 17.5 mmol). After 2 h at 25° C., the reaction was poured into diethyl ether (300 mL) and washed with water (1×100 mL), 1 N HCl (1×100 mL), water (1×100 mL), brine (1×50 mL) and dried over anhydrous $MgSO_4$. Crude compound 204A was analyzed by LCMS and NMR and determined to be pure enough to be carried on directly to the next step. HPLC: 100% at 4.347 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm).

B. (3aα,4β,7β,7aα)-4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]hexahydro-7-methyl-4,7-epoxy-1H-isobenzofuran-1,3(2H)-dione (204B)



Compound 204A (4.0 g, 18.9 mmol) and maleic anhydride (1.42 g, 14.5 mmol) were dissolved in dichloroethane (10 mL) and stirred at 25° C. for 60 hours. The volatiles were then removed in vacuo and the resulting orange oil was dissolved in absolute ethanol (50 mL) and 10% Pd/C (1.00 g, cat.) was added. Hydrogen was then introduced via a balloon. After 3 h, the reaction was filtered through Celite rinsing with EtOAc and concentrated in vacuo. The crude anhydride was purified by rapid flash chromatography on SiO_2 eluting with acetone/chloroform (0–2–4% acetone) to give 1.30 g (3.82 mmol, 20%) of compound 204B as a clear oil, in addition to 3.00 g (12.5 mmol, 66%) of the starting compound 204A. Characterization by proton NMR spectroscopy showed only the exo isomer. 1H NMR (400 MHz, $CDCl_3$) δ=3.83 (2H, t, J=6.0 Hz), 3.22 (1H, d, J=8.2 Hz), 3.06 (1H, d, J=8.2 Hz), 1.70–2.25 (6H, m), 1.55 (3H, s), 0.82 (9H, s), 0.00 (6H, s).

C. (3aα,4β,7β,7aα)-4-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]-oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (204C)



Compound 204B (0.250 g, 0.734 mmol) and 4-amino-2-trifluoromethyl-benzonitrile (0.124 g, 0.668 mmol) were suspended in dry toluene (2.0 mL) in a sealed tube. $MgSO_4$ (0.200 g) and triethylamine (0.5 mL) were then added and the tube was sealed and placed in a oil bath at 125° C. After 40 h, the reaction was cooled to 25° C., filtered and concentrated in vacuo. The crude material was purified by flash chromatography on SiO_2 eluting with CH_2Cl_2 to give 0.111 g (0.281 mmol, 30%) of compound 204C as a yellow solid. HPLC: 92% at 4.203 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 531.1 $[M+Na]^+$.

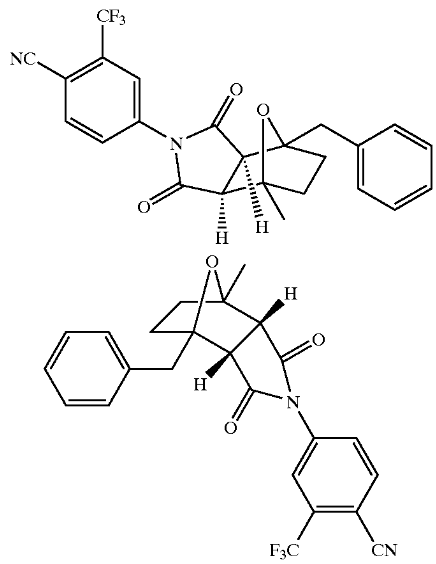
D. (3aα,4β,7β,7aα)-4-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (204D)

Compound 204C (0.031 g, 0.061 mmol) was dissolved in THF (0.5 mL) and transferred to a polypropylene container followed by cooling to 0° C. HF.pyridine (~47% HF, 0.1

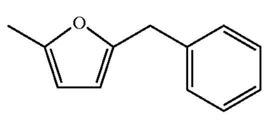
mL) was then added. After 15 min, the reaction was complete as determined by LC and was poured into cold sat. aqueous $NaHCO_3$. The mixture was extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were washed with 1 N HCl (1×20 mL) and dried over anhydrous Na_2SO_4. Compound 204D was isolated as a yellow oil and compared to the material prepared in Example 25. No purification was necessary.

EXAMPLE 205

(3aα,4β,7β,7aα)- and (3aα,4α,7α,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-(phenylmethyl)-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (205Ci and 205Cii, Respectively)

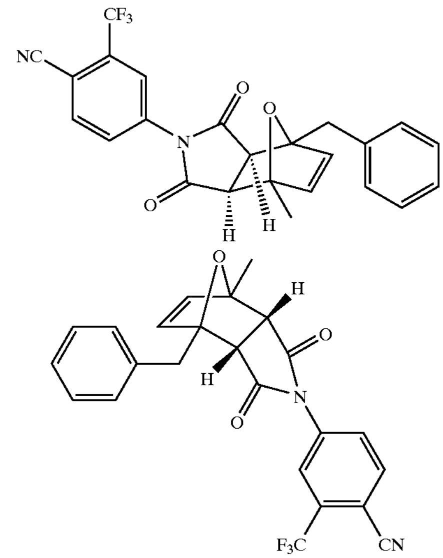


A. 2-Methyl-5-(phenylmethyl)-furan (205A)



n-BuLi (1.8 mL, 4.51 mmol, 2.5 M in hexane) was added to a solution of 2-methylfuran (0.37 mL, 4.10 mmol) in anhydrous THF (3 mL) at −25° C. The resulting solution was stirred at room temperature for 3 h and then cooled to −15° C. Benzyl bromide (0.59 mL, 4.92 mmol), which was passed through a plug of aluminum oxide, was added and the solution was warmed to rt and stirred overnight. Saturated NH_4Cl solution (5 mL) was added and the mixture was stirred for 1 h. The reaction mixture was then extracted with ether (2×5 mL) and the combined organic extracts were dried and concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with hexanes gave 323 mg (1.88 mmol, 46%) of compound 205A as colorless oil. HPLC: 95% at 3.72 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm) and about 400 mg mixture of product and benzyl bromide (~2:1 by HPLC).

B. (3aα,4β,7β,7aα)- and (3aα,4α,7α,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-(phenylmethyl)-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (205Bi and 205Bii, Respectively)



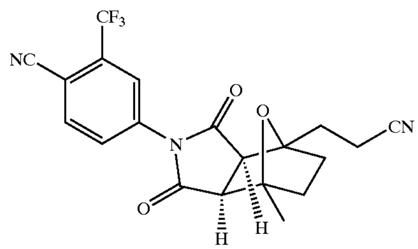
A solution of compound 205A (124 mg, 0.72 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (290 mg, 1.09 mmol) in CH_2Cl_2 (2 mL) was stirred at room temperature. After 4 days, the reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with CH_2Cl_2 gave 62 mg (0.14 mmol, 20%) of a mixture of compounds 205Bi and 205Bii as a white solid, which was used directly in the next step. HPLC: 93% at 3.69 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

C. (3aα,4β,7β,7aα)- and (3aα,4α,7α,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-(phenylmethyl)-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (205Ci and 205Cii, Respectively)

A solution of a mixture of compounds 205Bi and 205Bii (62 mg, 0.14 mmol) and 10% Pd/C (12 mg, cat.) in EtOH (3.5 mL) was stirred under a hydrogen atmosphere at room temperature for 2 h. The reaction mixture was filtered through Celite and concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 35% EtOAc/hexanes gave 22 mg (0.05 mmol, 35%) of compound 205Ci and 12 mg (0.027 mmols, 19%) of compound 205Cii. Compound 205Cii: HPLC: 98% at 3.75 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 458.2 $[M+NH_4]^+$. Compound 205Cii: HPLC: 97% at 3.78 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 473.45 $[M+CH_3OH]^+$.

EXAMPLE 206

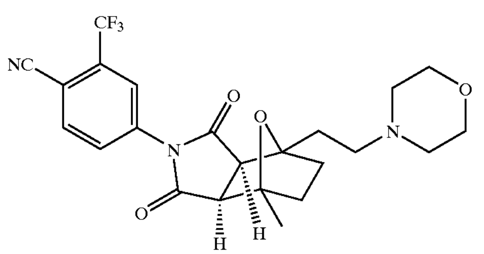
(3aα,4β,7β,7aα)-2-[4-Cyano-3-(trifluoromethyl)phenyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindole-4-propanenitrile (206)



A solution of compound 36 (34 mg, 0.074 mmol) and NaCN (24 mg, 0.49 mmol) in DMSO (1 mL) was heated at 100° C. for 0.5 h. After cooling, the reaction mixture was poured into H_2O (5 mL) and the aqueous layer was extracted with EtOAc (2×5 mL). The combined organic layers were washed with H_2O (2×5 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography on SiO_2 eluting with 50% EtOAc/hexanes followed by reverse phase preparative HPLC [30.41 min (retention time) (YMC S5 ODS 30×250 mm, 10–90% aqueous methanol over 30 minutes containing 0.1% TFA, 25 mL/min, monitoring at 220 nm)] gave 6.6 mg (0.016 mmol, 22%) of compound 206 as a white solid. HPLC: 99% at 2.89 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 402.1 $[M-H]^-$.

EXAMPLE 207

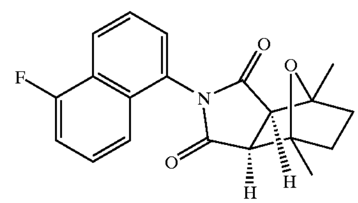
(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-7-[2-(4-morpholinyl)ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile, Trifluoroacetate (1:1) (207)



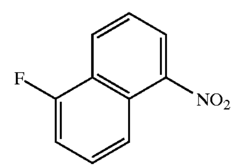
A solution of compound 36 (15.6 mg, 0.0341 mmol) and morpholine (6.0 μL, 0.068 mmol) in toluene (1 mL) was heated at 100° C. overnight. After cooling, the reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on SiO_2 eluting with 10% $MeOH/CH_2Cl_2$ followed by reverse phase preparative HPLC [23.96 min (retention time) (YMC S5 ODS 30×250 mm, 10–90% aqueous methanol over 30 minutes containing 0.1% TFA, 25 mL/min, monitoring at 220 nm)] gave 8.7 mg (0.015 mmol, 44%) of compound 207 (TFA salt) as a white solid. HPLC: 99% at 2.02 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 464.3 $[M+H]^+$.

EXAMPLE 208

(3aα,4β,7β,7aα)-2-(5-Fluoro-1-naphthalenyl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (208C)



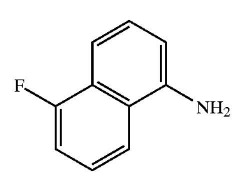
A. 1-Fluoro-5-nitronaphthalene (208A)



To a solution of 6 N HCl (12 mL) was added 1.47 g (7.83 mmol) of finely powdered 5-nitro-1-naphthylamine, as described in *J. Chem. Soc.* 1187 (1949). The mixture was cooled to 0° C. and a cold solution of $NaNO_2$ (547 mg, 7.93 mmol) in 2 mL H_2O was added slowly so that the temperature was kept near 0° C. After the addition was complete, the reaction mixture was stirred for 30 min and filtered. The filtrate was cooled to 0° C. and treated with cold 4.5 M $NaBF_4$ solution (5 mL) to give complete precipitation of the diazonium borofluoride. The mixture was kept at 0° C. for 30 min before it was filtered and the precipitates were washed with cold 4.5 M $NaBF_4$ solution (5 mL), ice-cold ethanol (10 mL) and Et_2O (20 mL). The obtained solids were air dried to yield 1.74 g (77%) of the corresponding diazonium salt.

To 1.70 g (5.92 mmol) of the above diazonium borofluoride was added 5 g of sand and the components were thoroughly mixed. The reaction mixture was heated cautiously under reduced pressure until decomposition set in. Toward the end of the reaction the flask was further heated for 30 min to 130° C. to assure complete conversion. After cooling the reaction mixture was dissolved in acetone and the contents were preabsorbed on silica gel. Purification was achieved by flash chromatography on silica gel, eluting with 0 to 10% EtOAc in hexanes to give 449 mg (2.35 mmol, 40%) of compound 208A as a white solid.

B. 1-Amino-5-fluoronaphthalene (208B)



A solution of compound 208A (62 mg, 0.32 mmol) in 1 mL EtOH containing 0.1 mL 12 N HCl was heated to reflux. Iron powder (62 mg, 1.11 mmol) was added in small portions and heating was continued for 2 h. The mixture was cooled, neutralized with 1 N NaOH solution and the aqueous

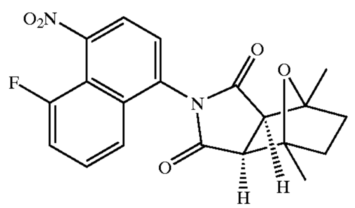
layer was extracted with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and concentrated in vacuo to leave a residue which was purified by flash chromatography on silica gel eluting with 40 to 80% EtOAc in hexanes to give 42 mg (0.26 mmol, 80%) of compound 208B as a yellow solid.

C. (3aα,4β,7β,7aα)-2-(5-Fluoro-1-naphthalenyl) hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3 (2H)-dione (208C)

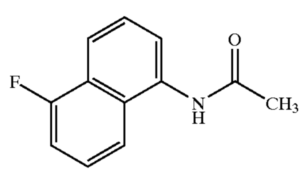
Compound 208B (42 mg, 0.26 mmol), compound 20A (54 mg, 0.27 mmol), $MgSO_4$ (69 mg, 0.58 mmol) and triethylamine (191 μL, 1.37 mmol) were taken up in 2 mL of toluene and placed in a sealed tube. The sealed tube was heated at 135° C. for 14 h. The cooled reaction mixture was filtered through a short pad of Celite eluting with CH_2Cl_2 and the solvent was removed under reduced pressure. The residue was purified by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm eluting with 30–100% aqueous methanol over 10 min containing 0.1% TFA, 20 mL/min) to give 15 mg (0.044 mmol, 17%) of compound 208C as a light yellow solid. HPLC: 16% at 2.96 min & 77% at 3.06 min (atropisomers, retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 340.2 $[M+H]^+$.

EXAMPLE 209

(3aα,4β,7β,7aα)-2-(5-Fluoro-4-nitro-1-naphthalenyl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (209C)

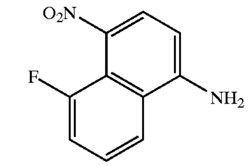


A. N-(5-Fluoro-1-naphthalenyl)acetamide (209A)



A solution of 141 mg (0.74 mmol) of compound 208A in 2 mL of AcOH was heated to reflux and treated with small portions of iron powder (118 mg, 2.11 mmol). The mixture was kept at reflux for 15 min before 73 μL (0.78 mmol) of Ac_2O was added. After an additional 15 min at reflux, the mixture was cooled and filtered eluting with CH_2Cl_2. The filtrate was then concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel eluting with 20 to 50% EtOAc in to give 145 mg (0.71 mmol, 97%) of compound 209A as a white solid.

B. 1-Amino-5-fluoro-4-nitronaphthalene (209B)



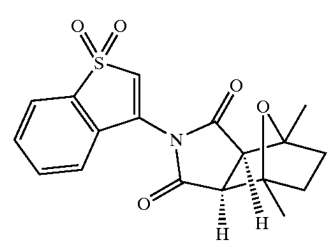
Compound 209A (133 mg, 0.645 mmol) was dissolved in 1 mL AcOH and the resulting solution was cooled to 10° C. At this temperature, 80.0 μL (2.00 mmol) of red fuming HNO_3 was added and stirring was continued for 15 min before the reaction was quenched by the addition of crushed ice. The aqueous layer was extracted with CH_2Cl_2 and the combined organic phases were dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was dissolved in 3 mL EtOH, heated to reflux and treated with 0.5 mL of 40% aqueous. NaOH solution. Stirring was continued for 15 min before the reaction was cooled and diluted with H_2O. The aqueous layer was extracted with CH_2Cl_2 and the combined organic phases were dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by flash chromatography on silica gel, eluting with 40 to 70% EtOAc in hexane to afford 36 mg (0.17 mmol, 27%) of compound 209B as a yellow solid.

C. 3aα,4β,7β,7aα)-2-(5-Fluoro-4-nitro-1-naphthalenyl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (209C)

Compound 209B (36 mg, 0.18 mmol) was reacted in a sealed tube with compound 20A (38 mg, 0.19 mmol), $MgSO_4$ (46 mg, 0.39 mmol) and Et_3N (128 μL, 0.920 mmol) in 250 μL toluene according to the above procedure described in example 208C to give, after purification by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm eluting with 30–100% aqueous methanol over 10 min containing 0.1% TFA, 20 mL/min), 27 mg (0.070 mmol, 39%) of compound 209C as a yellow solid. HPLC: 8% at 2.88 min & 84% at 3.06 min (atropisomers, retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 402.0 $[M+H]^+$.

EXAMPLE 210

(3aα,4β,7β,7aα)-2-(1,1-Dioxidobenzo[b]thiophen-3-yl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (210)

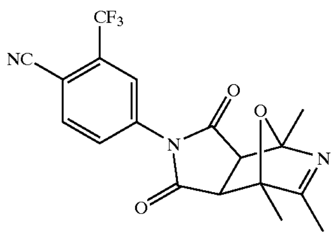


mCPBA (160 mg, 0.641 mmol, 70% pure) was added to a solution of compound 134 (70.0 mg, 0.214 mmol) in CH_2Cl_2 (2 mL) at rt. After the starting material was consumed, the reaction was quenched with sat. $NaHCO_3$,

and extracted with CH_2Cl_2. The organic layer was washed with 1 N NaOH, dried over Na_2SO_4 and concentrated under reduced pressure to give 63.9 mg (0.178 mmol, 83%) of compound 210 as a white solid. HPLC: 99% at 3.81 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 360.0 $[M+H]^+$.

EXAMPLE 211

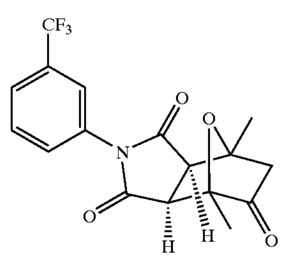
4-(1,3,3a,4,7,7a-Hexahydro-4,6,7-trimethyl-1,3-dioxo-4,7-epoxy-2H-pyrrolo[3,4-c]pyridin-2-yl)-2-(trifluoromethyl)benzonitrile (211)



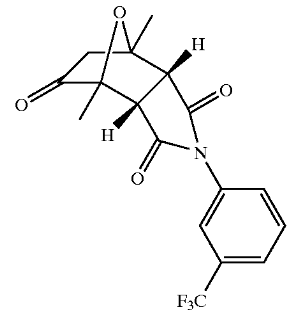
2,4,5-Trimethyl oxazole (0.48 mL, 4.14 mmol) was dissolved in toluene (2.0 mL) and 4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (1.00 g, 3.76 mmol) was added. The reaction mixture was stirred at 75° C. under nitrogen for 2.5 hrs. The solution was cooled to room temperature and the resulting precipitate was filtered and rinsed with toluene to give 0.51 g (35%) of compound 211 as a light grey solid. NMR analysis revelaed that compound 211 was one isomer (exo/endo) however the identity of the isomer could not be determined by NMR analysis. HPLC: 100% at 2.85 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 378.42 $[M+H]^+$.

EXAMPLE 212

(3aα,4β,7β,7aα)-Tetrahydro-4,7-dimethyl-2-[3-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3,5(2H,4H)-trione & (3aα,4α,7α,7aα)-Tetrahydro-4,7-dimethyl-2-[3-(trifluoromethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3,5(2H,4H)-trione (212i & 212ii, Respectively)



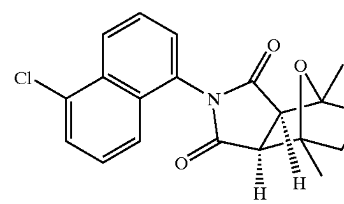
-continued



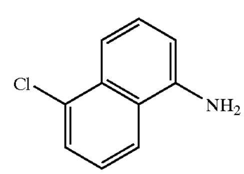
2,2-Dimethyl-3(H)-furanone (0.500 g, 4.46 mmol) and 1-[3-(trifluoromethyl)phenyl]-1H-pyrrole-2,5-dione (1.07 g, 4.46 mmol, prepared as described in Example 1B) were suspended in toluene (20 mL) in a sealed tube. The mixture was heated at 110° C. for 4 h and then cooled to 25° C. followed by concentration in vacuo. The resulting residue was purified by flash chromatography on SiO_2 eluting with methylene chloride to yield 0.411 g (26%) of compound 212i as a white solid and 0.193 g (12%) of compound 212ii as a white solid. The structural assignments were confirmed by. 1-D NOE proton NMR experiments. Compound 212i: HPLC: 100% at 2.817 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 376.0 $[M+Na]^+$. Compound 212ii: HPLC: 100% at 3.013 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 354.02 $[M+H]^+$.

EXAMPLE 213

(3aα,4β,7β,7aα)-2-(5-Chloro-1-naphthalenyl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (213B)



A. 1-Amino-5-chloronaphthalene (213A)



To a solution of 1.74 g (6.06 mmol) of the diazonium borofluoride (described in Example 208A) in acetone (7 mL) was added 693 mg (7.00 mmol) of CuCl in small portions. After the evolution of nitrogen had ceased the acetone was removed under reduced pressure and the residue was taken up in CH_2Cl_2 (30 mL). The organic phase was washed with H_2O (30 mL), dried over $MgSO_4$, concentrated in vacuo and

finally purified by flash chromatography (silica gel, EtOAc in hexane 0 to 15%) to give 754 mg (70%) of 1-chloro-5-nitronaphthalene.

The above synthesized 1-chloro-5-nitronaphthalene (540 mg, 2.6 mmol) was dissolved in 10 mL AcOH, followed by treatment with 415 mg (7.43 mmol) iron powder and subsequently acylated with Ac_2O (0.26 mL, 2.73 mmol) according to the procedure described in Example 209A to give 543 mg (95%) of 1-acetamino-5-chloronaphthalene.

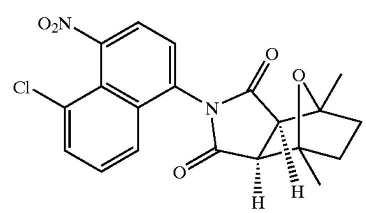
A solution of the above synthesized 1-acetamino-5-chloronaphthalene (52 mg, 0.24 mmol) in 3 mL EtOH was heated to reflux and treated with 0.5 mL 40% aqueous NaOH solution. The mixture was refluxed until no more starting material could be detected, cooled and concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (50 mL) and was washed with H_2O (25 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to leave 41 mg (98%) of compound 213A as a white solid.

B. (3aα,4β,7β,7aα)-2-(5-Chloro-1-naphthalenyl) hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3 (2H)-dione (213B)

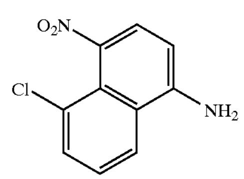
Compound 213A (24 mg, 0.14 mmol) was reacted in a sealed tube with compound 20A (29 mg, 0.15 mmol), $MgSO_4$ (36 mg, 0.30 mmol) and Et_3N (100 μL, 0.710 mmol) in 250 μL toluene according to the procedure described in Example 208C to give, after purification by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm eluting with 30–100% aqueous methanol over 10 min containing 0.1% TFA, 20 mL/min), 27 mg (40%) of compound 213B as a white solid. HPLC: 98% at 1.82 min (retention time) (YMC S5 TurboPack Pro column 4.6×33 mm eluting with 10–90% aqueous methanol over 2 minutes containing 0.2% phosphoric acid, 4 ml/min, monitoring at 220 nm). MS (ES): m/z 356.4 $[M+H]^+$.

EXAMPLE 214

(3aα,4β,7β,7aα)-2-(5-Chloro-4-nitro-1-naphthalenyl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (214B)



A. 1-Amino-5-chloro-4-nitronaphthalene (214A)



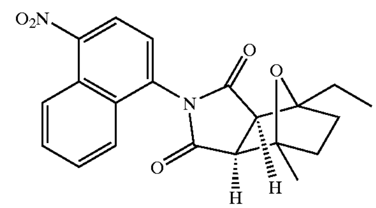
1-Acetamino-5-chloronaphthalene (150 mg, 0.68 mmol, prepared as described in Example 213A) was dissolved in 1 mL AcOH and treated with 82 μL of red fuming HNO_3 and subsequently deacylated with 1 mL 40% aqueous NaOH solution in 3 mL EtOH according to the procedure described in Example 209A to yield 49 mg (32%) of compound 214A as a yellow solid.

B. (3aα,4β,7β,7aα)-2-(5-Chloro-4-nitro-1-naphthalenyl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (214B)

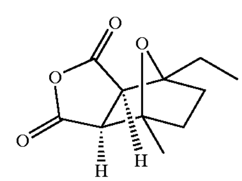
Compound 214A (27 mg, 0.12 mmol) was reacted in a sealed tube with compound 20A (26 mg, 0.13 mmol), $MgSO_4$ (32 mg, 0.27 mmol) and Et_3N (88 μL, 0.63 mmol) in 250 μL toluene according to the procedure described in Example 208C to give, after purification by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm eluting with 30–100% aqueous methanol over 10 min containing 0.1% TFA, 20 mL/min) 22 mg (45%) of compound 214B as a yellow solid. HPLC: 24% at 3.06 min & 76% at 3.25 min (atropisomers, retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 418.0 $[M+NH_4]^+$.

EXAMPLE 215

(3aα,4β,7β,7aα)-4-Ethylhexahydro-7-methyl-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3 (2H)-dione (215B)



A. (3aα,4β,7β,7aα)-4-Ethylhexahydro-7-methyl-4,7-epoxyisobenzofuran-1,3-dione (215A)



2-Ethyl-5-methylfuran (1.89 mL, 15.3 mmol) was dissolved in methylene chloride (10 mL) and maleic anhydride (1.00 g, 10.2 mmol) was added. The reaction was stirred at 25° C. for 18 h and then concentrated in vacuo. The resulting crude bicycle was dissolved in EtOAc (50 mL) and 10% Pd/C (0.40 g) was added. Hydrogen was then introduced via a balloon. After 4 h, the reaction was filtered through Celite, rinsing with EtOAc. Concentration in vacuo gave the crude compound 215A (1.93 g) as a white solid. This material was taken on directly to the next reaction without purification.

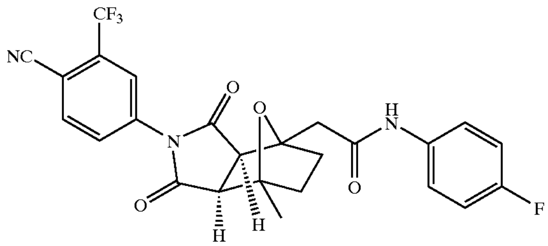
B. (3aα,4β,7β,7aα)-4-Ethylhexahydro-7-methyl-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3 (2H)-dione (215B)

Compound 215A (0.168 g, 0.798 mmol) and 1-amino-4-nitronaphthalene (0.10 g, 0.53 mmol) were suspended in toluene (0.8 mL) and TEA (0.2 mL) and magnesim sulfate (0.1 g) were added. The mixture was heated at 135° C. in a sealed tube for 18 h. The reaction was then cooled to rt and

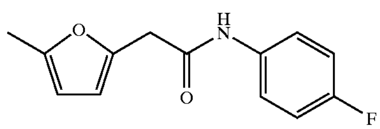
filtered, rinsing with chloroform. Concentration gave the crude product which was purified by preparative TLC on SiO_2 eluting with methylene chloride. This gave 0.077 g (0.20 mmol, 38%) of compound 215B as a yellow solid. HPLC: 100% at 3.260 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 381.05 $[M+H]^+$.

EXAMPLE 216

(3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl)-N-(4-fluorophenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindole-4-acetamide (216B)



A. N-(4-Fluorophenyl)-5-methyl-2-furanacetamide (216A)



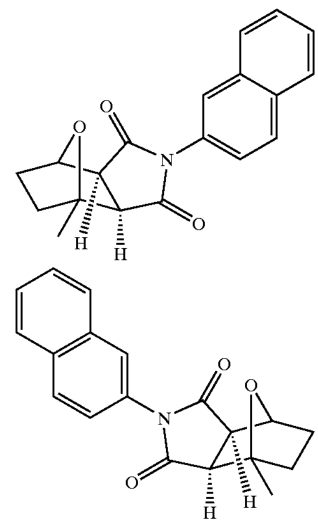
5-Methyl-2-furanacetic acid (1.00 g, 7.14 mmol, synthesized as described WO 9507893, Example 19) was dissolved in CH_3CN/DMF (4:1, 25 mL), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (1.37 g, 7.14 mmol) and 1-hydroxy-7-azabenzotriazole (0.972 g, 7.14 mmol) were then added followed by 4-fluoroaniline (0.676 mL, 7.14 mmol). After 3 h, the reaction was diluted with EtOAc (150 mL) and washed with 1 N HCl (1×30 mL), sat. aq. $NaHCO_3$ (1×30 mL), brine (1×40 mL) and dried over sodium sulfate. Compound 216A (1.58 g, 95%)) was isolated as a yellow foam after concentration in vacuo. No further purification was necessary. HPLC: 78% at 2.647 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

B. 3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl)-N-(4-fluorophenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindole-4-acetamide (216B)

Compound 216A (0.200 g, 0.858 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (0.164 g, 0.616 mmol) were dissolved in benzene and heated at 60° C. for 14 h. The reaction was then cooled and concentrated in vacuo. The resulting orange oil was dissolved in EtOAc (15 mL) and 10% Pd/C (0.050 g) was added. Hydrogen was then introduced via a balloon. After 3 h, the reaction was filtered through Celite rinsing with EtOAc and concentrated in vacuo. The resulting crude material was purified by preparative TLC on silica eluting with 5% acetone in methylene chloride to give 0.166 g (54%) of compound 216B as a white solid. NMR spectroscopy showed only a single isomer which was determined to be exo by NOE experiments. HPLC: 95% at 3.200 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 484.0 $[M+H]^+$.

EXAMPLE 217

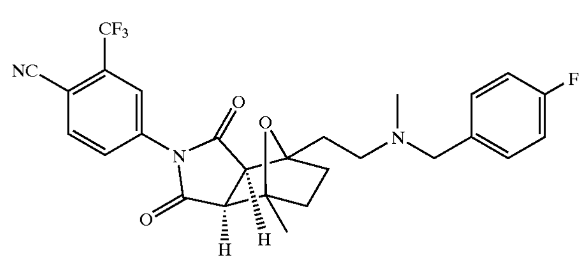
(3aα,4β,7β,7aα)-Hexahydro-4-methyl-2-(2-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione, Faster Eluting Enantiomer & (3aα,4β,7β,7aα)-Hexahydro-4-methyl-2-(2-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione, Slower Eluting Enantiomer (217i & 217ii, Respectively)



Racemic compound 137 was separated into the individual antipodes by chiral reverse phase liquid chromatography. A Chiralpak AD-R column (4.6×250 mm) was used eluting with 70% acetonitrile/30% water at 1 mL/min. UV detection at 220 nm was used. The faster eluting isomer, compound 217i (retention time=15.66 min), was found to be 99.9% ee and the slower eluting isomer, compound 217ii (retention time=15.66 min) was 99.6% ee by analytical chiral reverse phase chromatography.

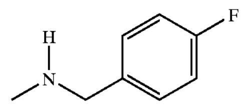
EXAMPLE 218

(3aα,4β,7β,7aα)-4-[4-[2-[[(4-Fluorophenyl)methyl]methylamino]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (218B)

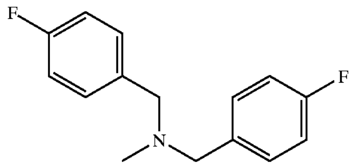


A. (4-Fluorobenzyl)methylamine & Bis(4-fluorobenzyl)methylamine (218A & 218A')

218A



218A'



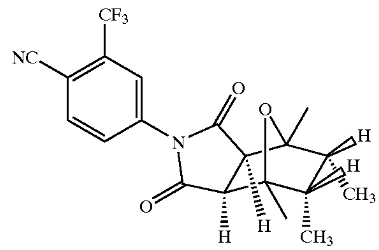
Compounds 218A & 218A' were made in accordance with the procedure described by Singer et al. *J. Med. Chem.* 29, 40–44 (1986). 4-Fluorobenzyl bromide (189 mg, 1.00 mmol) was refluxed in a solution of ethanol (1.5 mL) and methylamine (5 mL, 2 M solution in MeOH) for 3 h. An additional portion of methylamine (2 mL) was added and the mixture was refluxed for an additional hour. The solution was cooled and concentrated in vacuo, and the residue was dissolved in a mixture of 2 N HCl (3 mL) and ether (1.5 mL). The layers were separated and the aqueous layer was extracted with an additional portion of ether. The aqueous solution was chilled to 0° C., titrated to pH 11 with NaOH and extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and concentrated in vacuo to give 120 mg of a 2.5:1 mixture of compounds 218A and compound 218A' respectively. The crude mixture was taken on without further purification.

B. (3aα,4β,7β,7aα)-4-[4-[2-[[(4-Fluorophenyl) methyl]-methylamino]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (218B)

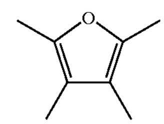
A solution of compound 36 (34.3 mg, 0.075 mmol) and compounds 218A & 218A' (21 mg, ~0.088 mmol (of 218A)) in toluene (0.4 mL) was heated at 100° C. overnight. The reaction mixture was cooled to room temperature and then concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 25% acetone/75% CH_2Cl_2 gave 30 mg (0.058 mmol, 78%) of 218B as a yellow solid. HPLC: 99% at 2.46 min (retention time) (YMC S5 ODS 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, monitoring at 220 nm). MS (ES): m/z 516.26 $[M+H]^+$.

EXAMPLE 219

(3aα,4β,5β,6β,7β,7aα)-4-(Octahydro-4,5,6,7-tetramethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (219D)



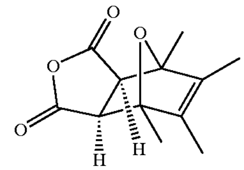
A. 2,3,4,5-Tetramethylfuran (219A)



Compound 219A was made in accordance with the procedures described in Hancock et al. *J. Org. Chem.* 42,1850–1856 (1977) & Amarnath et al. *J. Org. Chem.,* 60, 301–307 (1995). 2-Propanone (100 mL, 1.1 mol) was refluxed over PbO_2 (26.7 g, 0.112 mol) for 28 h. After cooling to rt, the reaction mixture was filtered and the residue was washed with acetone. The filtrate was concentrated under reduced pressure to remove the acetone and then distilled at 20 Torr. The fraction that came over between 100–120° C. was collected to give 6.75 g (42.5%) of 3,4-dimethylhexane-2,5-dione as a light yellow oil.

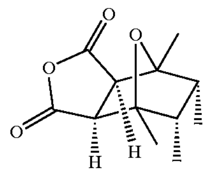
A solution of 3,4-dimethylhexane-2,5-dione (3.00 g, 21.1 mmol) and p-toluenesulfonic acid (401 mg, 2.11 mmol) in benzene (30 mL) was heated to reflux in a Dean-Stark trap overnight. The reaction mixture was distilled at atmospheric pressure to remove the excess benzene. The remaining mixture was transferred to a smaller flask and distilled at atmospheric pressure. The fraction that came over between 80–100° C. was collected to give 509 mg (19%) of compound 219A as a light yellow oil.

B. (3aα,4β,7β,7aα)-4-Ethyl-3a,4,7,7a-tetrahydro-4,5,6,7-tetramethyl-4,7-epoxyisobenzofuran-1,3-dione (219B)



A solution of compound 219A (400 mg, 3.22 mmol) and maleic anhydride (442 mg, 4.51 mmol) in Et_2O (1.5 mL) was stirred at rt overnight. The reaction mixture was then placed in freezer for 5 days, after which time the resulting crystals were collected and dried to give 0.26 g (37%) of compound 219B as tan crystals. The crude compound 219B was taken on to the next step without further purification.

C. (3aα,4β,5α,6α,7β,7aα)-4-Ethylhexahydro-4,5,6,7-tetramethyl-4,7-epoxyisobenzofuran-1,3-dione (219C)



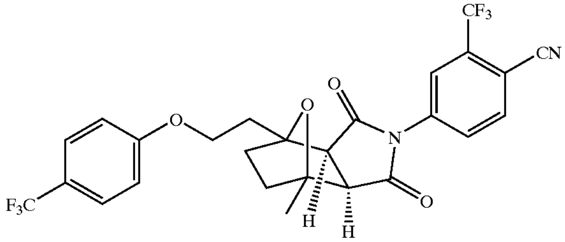
A solution of compound 219B (120 mg, 0.545 mmol) and 10% Pd/C (24 mg, cat.) in EtOAc (2 mL) was stirred under a balloon of hydrogen at room temperature overnight. The reaction mixture was filtered through Celite and concentrated under reduced pressure to give 100 mg (0.446 mmol, 82%) of compound 219C as a white solid, which was carried on with no further purification.

D. (3aα,4β,5β,6β,7β,7aα)-4-(Octahydro-4,5,6,7-tetramethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (219D)

A solution of compound 219C (44.4 mg, 0.2 mmol), 5-amino-2-cyanobenzotrifluoride (45 mg, 0.24 mmol), TEA (0.04 mL) and $MgSO_4$ (20 mg) in toluene (0.2 mL) was heated at 135° C. overnight. The reaction mixture was cooled to room temperature, filtered and then concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 40% EtOAc/hexanes followed by washing the resulting solid with MeOH gave 17 mg (0.043 mmol, 22%) of compound 219D as a white solid. HPLC: 90% at 3.11 min (retention time) (YMC S5 ODS 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, monitoring at 220 nm). MS (ES): m/z 391.2 $[M-H]^-$.

EXAMPLE 220

(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[4-(trifluoromethyl)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile, Faster Eluting Antipode & (3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[4-(trifluoromethyl)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile, Slower Eluting Enantiomer (220i & 220ii, Respectively)



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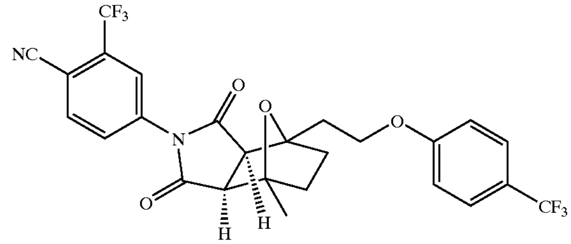
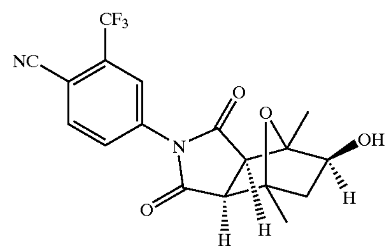


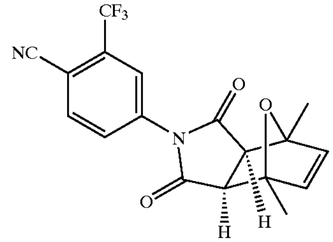
Racemic compound 35 was separated into the individual antipodes by chiral normal phase liquid chromatography. A Chiralpak AD column (50×500 mm) was used eluting with 85% hexanes/7.5% methanol/7.5% ethanol, at 50 mL/min. UV detection at 220 nm was used. The faster eluting isomer compound 220i (retention time=55.86 min) was found to have 95.8% ee ($[\alpha]_D^{25}$=−53.02°, C=3.134 mg/cc in CH_2Cl_2) and the slower eluting isomer compound 220ii (retention time=62.86 min) was 86% ee ($[\alpha]_D^{25}$=+48.74°, C=2.242 mg/cc in CH_2Cl_2) by analytical chiral normal phase chromatography.

EXAMPLE 221

(3aα,4β,5β,7β,7aα)-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (221B)



A. (3aα,4β,7β,7aα)-4-(hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (221Ai) & (3aα,4α,7α,7aα)-4-(hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (221Aii)



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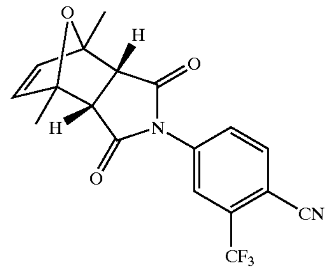


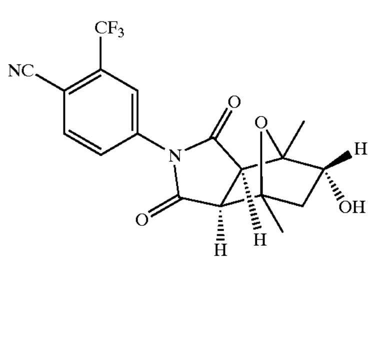
A solution of 2,5-dimethylfuran (0.800 mL, 7.51 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (synthesized as described in Example 1B, using 4-cyano-3-trifluoromethylaniline in place of 4-bromo-3-methylaniline) (1.00 g, 3.75 mmol) in benzene (4 mL) was heated at 60° C. overnight. The reaction mixture was concentrated under reduced pressure and placed on a high vacuum pump until the oil solidified to give a 3:1 mixture (determined by LC and NMR) of compounds 221Ai & 221Aii, respectively, as a brown solid, which was used directly in the next step without further purification.

B. (3aα,4β,5β,7β,7aα)-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (221B)

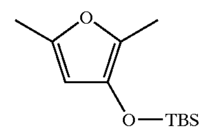
BH_3.THF (3.75 mL, 3.75 mmol, 1M in THF) was added to a solution of crude compounds 221Ai & 221Aii (3.75 mmol) in THF (12.5 mL) at 0° C. After the starting material was consumed the reaction mixture was concentrated under reduced pressure. The resulting residue was then dissolved in toluene (12.5 mL), Me_3NO (845 mg, 11.2 mmol) was added and the mixture was heated to reflux overnight. The reaction mixture was then cooled to rt, added to H_2O and extracted with EtOAc (3×). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by flash chromatography on SiO_2 eluting with 75% EtOAc/hexanes gave 0.354 g (25%) of compound 221B as a tan powder. HPLC: 90% at 2.45 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 381.11 $[M+H]^+$.

EXAMPLE 222

(3aα,4β,5α,7β,7aα)-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (222D)

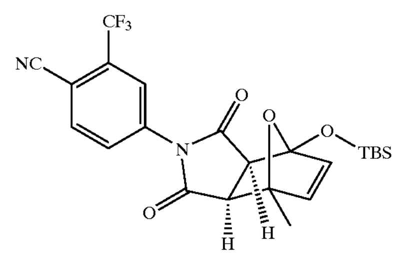


A. 3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-2,5-dimethylfuran (222A)



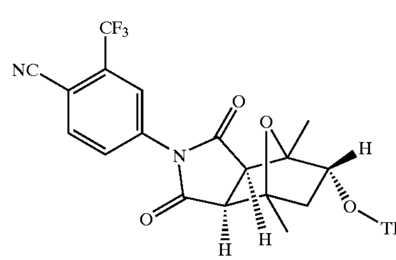
2,5-Dimethyl-3(3H)-furanone (2.00 g, 17.8 mmol) was dissolved in methylene chloride (180 mL). TEA (7.43 mL, 53.5 mmol) was added followed by TBSOTf(4.92 mL, 21.4 mmol) at 25° C. After 1 h, the reaction was concentrated in vacuo and the resulting slurry was run through a silica gel column conditioned with 3% TEA in hexanes. The product was eluted with 3% TEA/hexanes to give 3.6 g (89%) of compound 222A as an orange oil which was used directly in subsequent reactions.

B. (3aα,4β,7β,7aα)-4-[5-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-1,3,3a,4,7,7a-hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (222B)



4-(2,5-Dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (1.00 g, 3.85 mmol) was dissolved in benzene (5.0 mL) and the compound 222A (1.30 g, 5.77 mmol) was added. The reaction mixture was warmed to 60° C. for 2 h and then cooled to 25° C. The solution was then concentrated in vacuo to give compound 222B as a yellow oil which was carried on to the next reaction without purification. HPLC: 60% at 4.013 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

C. (3aα,4β,5α,7β,7aα)-4-[5-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (222C)



Crude compound 222B (3.85 mmol) was dissolved in ethyl acetate (75 mL) and 10% Pd/C (1.20 g) was added. Hydrogen was then introduced via a balloon. After 24 h, the

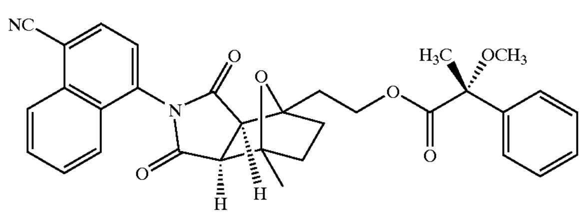
reaction was filtered through Celite rinsing with ethyl acetate and concentrated in vacuo to give a yellow oil. The crude product was purified by flash chromatography on silica gel eluting with methylene chloride/acetone (0%–1%–2% acetone) to give 0.710 g (35%) compound 222C as a yellow solid. HPLC: 100% at 4.160 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 517.6 $[M+Na]^+$.

D. (3aα,4β,5α,7β,7aα)-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (222D)

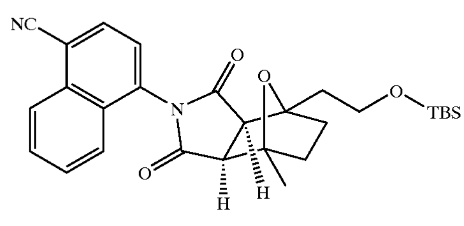
Compound 222C (0.040 g, 0.081 mmol) was dissolved in THF (1.0 mL) and HF.Pyridine (0.5 mL) was added. After 2 h, the reaction was carefully poured into cold saturated aq. $NaHCO_3$. The mixture was then extracted with methylene chloride (3×10 mL). The combined organics were washed with 1 N HCl (1×10 mL) and dried over anhydrous sodium sulfate. Concentration in vacuo gave 0.031 g (10%) compound 222D as a yellow solid. NOE experiments confirmed the assigned isomer. HPLC: 98% at 2.777 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 403.06 $[M+Na]^+$.

EXAMPLE 223

(αR)-α-Methoxybenzeneacetic Acid, 2-[(3aα,4β,7β,7aα)-2-(4-cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-y]ethyl Ester (223C)



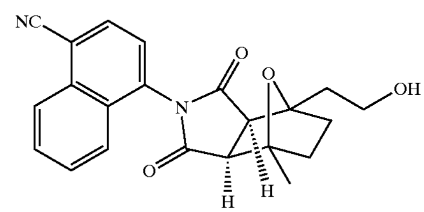
A. (3aα,4β,7β,7aα)-4-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (223A)



A solution of 4-amino-1-naphthalenecarbonitrile (19.2 g, 114 mmol) and maleic anhydride (14.0 g, 113 mmol) in AcOH (230 mL) was heated at 115° C. for 12 h. After cooling to rt, the reaction mixture was concentrated under reduced pressure then diluted with CH_2Cl_2 (2.5 L). The organic layer was washed 3× with H_2O (3 L), 1× with sat. aq. Na_2CO_3 (1 L) and 1× with brine (1 L), dried over $MgSO_4$ and concentrated to ~200 mL under reduced pressure. Purification by flash chromatography on cation exchange resin (60 g, CUBX13M6 from United Chemical Technologies) eluting with CH_2Cl_2 gave 25.0 g (88%) of 4-(2,5-dihydro-2,5-dioxo-1H-1-yl)-1-naphthalenecarbonitrile as a yellow solid. HPLC: 96% at 2.48 min (retention time) (Phenomenex-prime S5-C18 column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 249.25 $[M+H]^+$.

4-(2,5-Dihydro-2,5-dioxo-1H-1-yl)-1-naphthalenecarbonitrile (1.00 g, 4.03 mmol) was suspended in benzene (6.0 mL) in a sealed tube and compound 204A (1.11 g, 5.24 mmol) was added. The reaction was heated at 60° C. for 16 h and then cooled to 25° C. The benzene was removed in vacuo to give a yellow solid. The solid was dissolved in ethyl acetate (40 mL) and Pd/C (10% Pd, 0.300 g) was added. Hydrogen was then introduced via a balloon. After 4 h, the reaction was filtered through Celite rinsing with ethyl acetate. Concentration in vacuo gave a pale yellow solid which was purified by flash chromatography on silica gel eluting with acetone/chloroform (0% –1.5%–3% acetone) to give 1.53 g (77%) compound 223A as a yellow foam. HPLC: 86% at 4.173 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

B. (3aα,4β,7β,7aα)-4-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (223B)



Compound 223A (1.37 g, 2.97 mmol) was dissolved in THF (8.0 mL) and transferred to a polypropylene bottle and cooled to 0° C. HF∘Pyridine (2.0 mL) was then added. After 20 min, the reaction was carefully poured into cold sat. aq. sodium bicarbonate and extracted with methylene chloride (3×30 mL). The organics were then washed with 1 N HCl and dried over anhydrous sodium sulfate. Concentration in vacuo gave 0.99 g (89%) the compound 223B as a yellow foam which was not purified further. HPLC: 96% at 2.443 and 2.597 min (atropisomers, retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 399.02 $[M+Na]^+$.

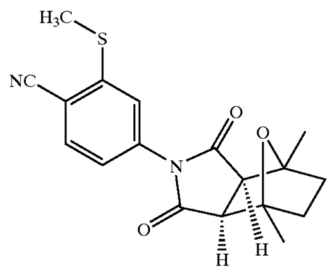
C. (αR)-α-Methoxybenzeneacetic Acid, 2-[(3aα,4β,7β,7aα)-2-(4-cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-y]ethyl Ester (223C)

Compound 223B (0.200 g, 0.575 mmol) was added to a solution of WSDCC (0.138 g, 0.719 mmol) and (R)-mandelic acid (0.096 g, 0.57 mmol) in dichloromethane (6.0 mL). 4-DMAP (0.005 g) was then added and the reaction was stirred at 25° C. for 4 h. The mixture was then diluted with dichloromethane, washed with 1 N HCl (2×10 mL) followed by sodium bicarbonate (1×10 mL) and dried over anhydrous sodium sulfate. Concentration in vacuo gave

0.220 g (71%) compound 223C as a yellow solid which was not purified further. HPLC: 100% at 3.283 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 547.26 $[M+Na]^+$.

EXAMPLE 224

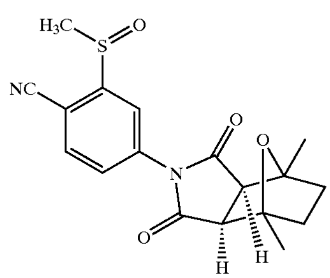
(3aα,4β,7β,7aα)-2-(Methylthio)-4-(octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl) benzonitrile (224)



4-Amino-2-(methylthio)benzonitrile (100 mg, 0.609 mmol, synthesized as described in EP 40931 A1) was reacted in a sealed tube with compound 20A (131 mg, 0.668 mmol), $MgSO_4$ (161 mg, 1.34 mmol) and Et_3N (0.440 mL, 3.17 mmol) in 0.50 mL toluene according to the procedure described in Example 208C to give, after purification by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm eluting with 30–100% aqueous methanol over 10 min containing 0.1% TFA, 20 mL/min), 137 mg (0.400 mmol, 66%) of compound 224 as a white solid. HPLC: 100% at 2.73 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 401.0 $[M-H+OAc]^-$.

EXAMPLE 225

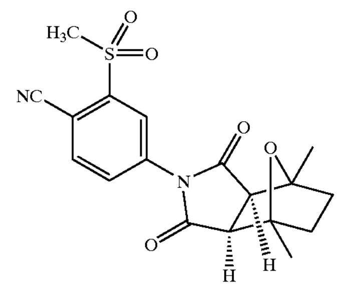
(3aα,4β,7β,7aα)-2-(Methylsulfilnyl)-4-(octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl) benzonitrile (225)



To an ice-cold suspension of compound 224 (30 mg, 0.088 mmol) in 2 mL of H_2O/MeOH (1:1) was added oxone (80 mg, 0.26 mmol) in one solid portion. The resulting mixture was stirred for 4 h at 0° C. before it was diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (2×20 mL). The combined organic layers were dried and concentrated in vacuo to leave a residue which was purified by filtering the material through a short pad of silica gel eluting with CH_2Cl_2 to yield 32 mg (0.088 mmol, 100%) of compound 225 as a colorless oil. HPLC: 99% at 2.01 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 376.0 $[M+NH_4]^+$.

EXAMPLE 226

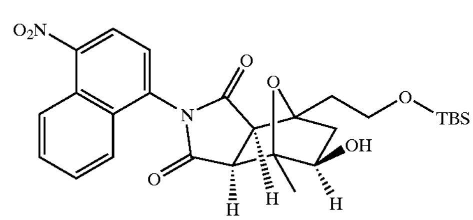
(3aα,4β,7β,7aα)-2-(Methylsulfonyl)-4-(octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl) benzonitrile (226)



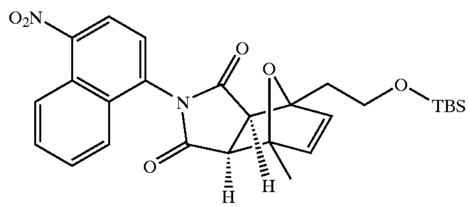
To a solution of compound 225 (48 mg, 0.14 mmol) in CH_2Cl_2 (2 mL) was added mCPBA (145 mg, 50% mixture, 0.420 mmol) in one solid portion. The resulting mixture was allowed to warm to room temperature and was stirred for 60 h at which time no more starting material could be detected by HPLC. The reaction was quenched by the addition of sat. $NaHCO_3$ solution (5 mL), the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (20 mL). The combined organic phases were dried over $MgSO_4$ and concentrated in vacuo. The remaining residue was purified by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm eluting with 30–100% aqueous methanol over 10 min containing 0.1% TFA, 20 mL/min) to afford 48 mg (0.13 mmol, 92%) of compound 226 as a white solid. HPLC: 100% at 2.07 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 392.0 $[M+NH_4]^+$.

EXAMPLE 227

(3aα,4β,5β,7β,7aα)-7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]hexahydro-5-hydroxy-4-methyl-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione (227B)



A. (3aα,4β,7β,7aα)-4-[2-[[(1,1-Dimethylethyl) dimethylsilyl]oxy]ethyl]-3a,4,7,7a-tetrahydro-7-methyl-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione (227A)



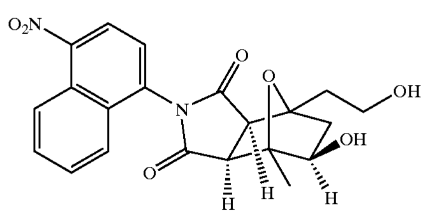
A solution of compound 204A (455 mg, 1.89 mmol) and 1-[4-nitronaphthalene-1H-pyrrole-2,5-dione (254 mg, 0.947 mmol, prepared as described for 4-(2,5-dihydro-2,5-dioxo-1H-1-yl)-1-naphthalenecarbonitrile, Example 223A) in benzene (2 mL) was heated at 60° C. overnight. The reaction mixture was concentrated under reduced pressure to give crude compound 227A as a brown solid, which was used directly in the next step without further purification.

B. (3aα,4β,5β,7β,7aα)-7-[2-[[(1,1-Dimethylethyl) dimethylsilyl]oxy]ethyl]hexahydro-5-hydroxy-4-methyl-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione (227B)

BH_3.THF (0.95 mL, 0.95 mmol, 1M in THF) was added to a solution of crude compound 227A (0.48 g, 0.95 mmol) in THF (2 mL) at 0° C. After compound 227A was consumed, as was evident by HPLC, the reaction mixture was concentrated under reduced pressure. The resulting residue was then dissolved in toluene (2 mL), Me_3NO (71.0 mg, 2.84 mmol) was added and the mixture was heated to reflux overnight. The reaction mixture was then cooled to rt, added to H_2O and extracted with EtOAc (3×). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by flash chromatography on SiO_2 eluting with 75% EtOAc/hexanes, gave 130 mg (26%) of compound 227B as a brown solid. HPLC: 94% at 3.92 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 527.5 $[M+H]^+$.

EXAMPLE 228

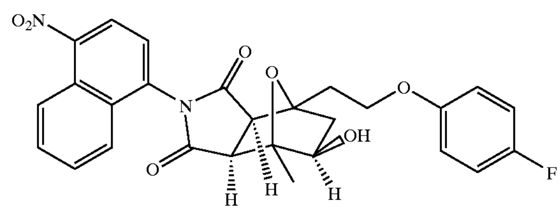
(3aα,4β,5β,7β,7aα)-Hexahydro-5-hydroxy-7-(2-hydroxyethyl)-4-methyl-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione (228)



A mixture of TBAF (0.3 mL, 0.3 mmol, 1 M solution in THF) and HF (0.3 mL, 50% in H_2O) in CH_3CN (6 mL) was added to a solution of 227B (104 mg, 0.197 mmol) in THF (2 mL) at 0° C. The reaction mixture was stirred overnight at rt. After the starting material was consumed, as was evident by TLC, H_2O and EtOAc were added and the layers were separated. The aqueous layer was extracted with EtOAc (1×) and the combined organic layers were washed with H_2O (1×) and brine (1×), dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography on SiO_2 eluting with 5% MeOH/CH_2Cl_2 gave 61 mg (75%) of compound 228 as a yellow solid. HPLC: 99% at 2.47 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 411.2 $[M-H]^-$.

EXAMPLE 229

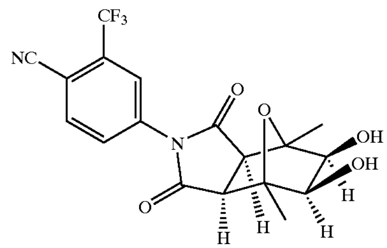
(3aα,4β,5β,7β,7aα)-7-[2-(4-Fluorophenoxy)ethyl] hexahydro-5-hydroxy-4-methyl-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione (229)

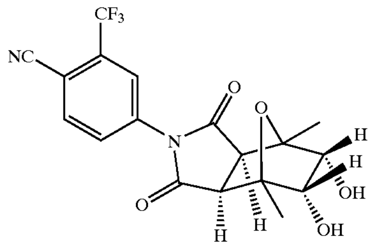


DBAD (37.7 mg, 0.164 mmol) was added to a solution of PPh_3 (43.0 mg, 0.164 mmol) in THF (1 mL). After stirring for 10 min, 4-fluorophenol (18.3 mg, 0.164 mmol) was added and the reaction mixture was stirred for a further 5 min. A solution of compound 228 (45.0 mg, 0.109 mmol) in THF (1 mL) was added and the mixture was stirred at rt overnight. HPLC showed the crude reaction mixture to contain mostly starting diol (compound 228), so this mixture was added to a preformed mixture as before of PPh_3 (86 mg), DBAD (75.4 mg) and phenol (36.6 mg) in THF (4 mL) at rt. Stirring was continued until all of compound 228 was consumed. The reaction was then concentrated under reduced pressure. Purification by reverse phase preparative HPLC [15.2 min (retention time) (YMC S5 ODS A column 20×100 mm, 10–90% aqueous methanol over 15 minutes containing 0.1% TFA, 20 mL/min, monitoring at 220 nm)] gave 25.0 mg (45%) of compound 229 as a light yellow solid. HPLC: 99% at 3.53 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 505.2 $[M-H]^-$.

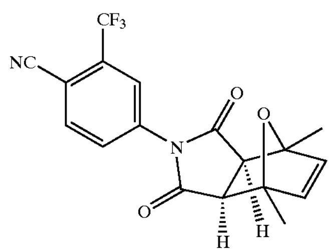
EXAMPLE 230

(3aα,4β,5β,6β,7β,7aα)-4-(Octahydro-5,6-dihydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile & (3aα,4β,5α,6α,7β,7aα)-4-(Octahydro-5,6-dihydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (230Bi & 230Bii. Respectively)





A. (3aα,4β,7β,7aα)-4-(1,3,3a,4,7,7a-Hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (230A)



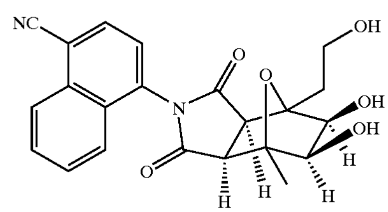
2,5-Dimethyl furan (1.23 mL, 11.5 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (2.00 g, 7.69 mmol) were dissolved in benzene (10 mL) and heated at 60° C. for 18 h. The volatile organics were then removed in vacuo. The resulting crude compound 230A was carried on without purification. HPLC: 71% at 3.007 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

B. (3aα,4β,5β,6β,7β,7aα)-4-(Octahydro-5,6-dihydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile & (3aα,4β,5α,6α,7β,7aα)-4-(Octahydro-5,6-dihydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (230Bi & 230Bii)

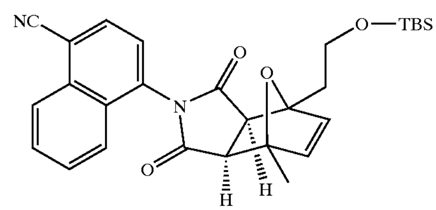
Compound 230A (0.100 g, 0.281 mmol) was dissolved in acetone and N-methylmorpholine-N-oxide (50% aq. solution, 0.10 mL, 0.42 mmol) was added. OsO_4 (4% aq. solution, 0.014 mmol) was then added. After 3 h at 25° C., the reaction was complete and sodium sulfite (0.250 g) was added with vigorous stirring. After 15 min, brine (10 mL) was added and the solution was extracted with EtOAc (3×15 mL). The organics were dried over anhydrous sodium sulfate and then concentrated in vacuo. The crude diol mixture was purified by preparative TLC eluting with 18% acetone in chloroform to give 0.038 g (34%) of compound 230Bi (beta face) and 0.012 g (11%) of compound 230Bii (alpha face) as pale yellow solids. Compound 230Bi: HPLC: 100% at 2.567 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 397.08 $[M+H]^+$. Compound 230Bii: HPLC: 100% at 2.417 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 397.08 $[M+H]^+$.

EXAMPLE 231

(3aα,4β,5β,6β,7β,7aα)-4-[Octahydro-5,6-dihydroxy-4-(hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (231C)

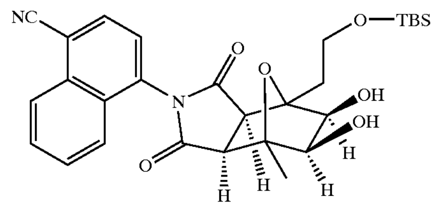


A. (3aα,4β,7β,7aα)-4-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-1,3,3a,4,7,7a-hexahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (231A)



Compound 204A (29.0 g, 120 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-1-yl)-1-naphthalenecarbonitrile (20.0 g, 80.6 mmol) were suspended in benzene (80 mL) and heated at 60° C. for 14 h. The mixture was then concentrated in vacuo at 40° C. for 40 min. The resulting slurry was cooled to 25° C. and then suspended in MeOH (200 mL) and stirred at rt for 30 min. The solution was then cooled, to 0° C. for 30 min and then filtered rinsing with cold MeOH. The resulting solid was dried in vacuo to give 26.1 g (55%) of crude compound 231A as a white solid. The methanol solution was concentrated in vacuo and resuspended in MeOH (50 mL) and cooled to −20° C. for 4 h. The solution was then filtered rinsing with cold MeOH. The resulting solid was dried in vacuo to give 3.8 g (10%) of compound 231A as a white solid. HPLC: 95% at 4.227 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

B. (3aα,4β,5β,6β,7β,7aα)-4-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-5,6-dihydroxy-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (231B)



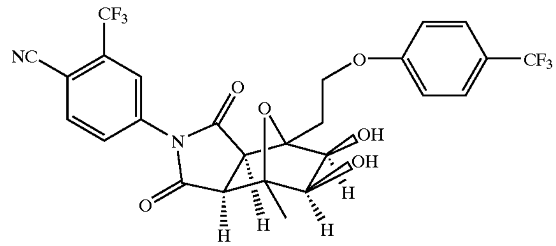
Compound 231A (0.400 g, 0.851 mmol) was dissolved in acetone (9.0 mL) and N-methylmorpholine-N-oxide (50% aq. solution, 0.150 mL, 1.28 mmol) was added. OsO_4 (4% aq. solution, 0.043 mmol) was then added. After 3 h at 25° C., the reaction was complete and sodium sulfite (1.0 g) was added with vigorous stirring. After 15 minutes, brine (30 mL) was added and the solution extracted with EtOAc (3×50 mL). The organics were dried over anhydrous sodium sulfate and then concentrated in vacuo. The crude diol was purified by flash chromatography on silica eluting with 5–25% acetone in chloroform to give 0.355 g (80%) of compound 231B as a yellow solid. HPLC: 93% at 3.903 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 522.00 $[M+H]^+$.

C. (3aα,4β,5β,6β,7β,7aα)-4-[Octahydro-5,6-dihydroxy-4-(hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (231C)

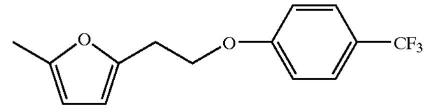
Compound 231B (0.400 g, 0.766 mmol) was dissolved in THF (5.0 mL) and transferred to a polypropylene bottle and cooled to 0° C. HF•Pyridine (1.0 mL) was then added. After 20 min, the reaction was carefully poured into cold sat. aq. sodium bicarbonate and extracted with methylene chloride (3×30 mL). The organics were then washed once with 1 N HCl and dried over anhydrous sodium sulfate. Concentration in vacuo gave 0.290 g (93%) compound 231C (0.290 g) as a yellow foam which was not purified further. HPLC: 92% at 2.273 and 2.423 min (atropisomers, retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 409.10 $[M+H]^+$.

EXAMPLE 232

(3aα,4β,5β,6β,7β,7aα)-4-[Octahydro-5,6-dihydroxy-4-methyl-1,3-dioxo-7-[2-[4-(trifluoromethyl)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile, (232C)

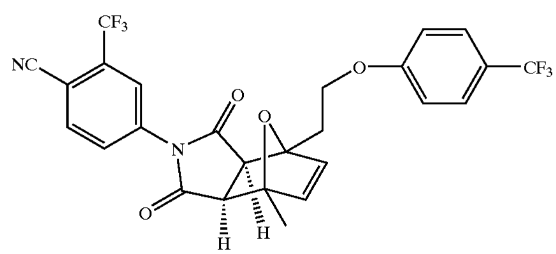


A. 2-Methyl-5-[2-[4-(trifluoromethyl)phenoxy]ethyl]furan (232A)



To a solution of triphenylphosphine (1.56 g, 5.95 mmol) in THF (40 mL) was added DBAD (1.37 g, 5.95 mmol). After 10 min, 4-trifluoromethylphenol (0.964 g, 5.95 mmol) was added. After 10 additional minutes, compound 21A (0.500 g, 3.97 mmol) was added. After 14 h at 25° C., the reaction was concentrated in vacuo and purified by flash chromatography on silica eluting with chloroform to give 0.713 g (44%) of compound 232A as a clear oil.

B. (3aα,4β,7β,7aα)-4-[1,3,3a,4,7,7a-hexahydro-4-methyl-1,3-dioxo-7-[2-[4-(trifluoromethyl)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (232B)



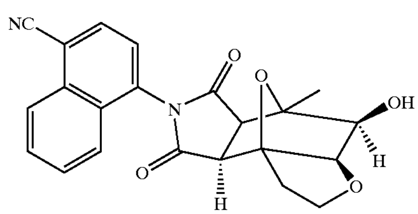
Compound 232A (0.301 g, 1.15 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (0.220 g, 0.846 mmol) were suspended in benzene (1.5 mL) and heated at 60° C. for 14 h. The mixture was then concentrated in vacuo at 40° C. for 40 minutes. The crude product was purified by flash chromatography on silica eluting with 10–0% hexanes in methylene chloride to give 0.199 g (44%) of compound 232B as a yellow solid. Compound 232B was characterized as the exo diastereomer by NOE experiments. HPLC: 94% at 3.993 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

C. (3aα,4β,5β,6β,7β,7aα)-4-[Octahydro-5,6-dihydroxy-4-methyl-1,3-dioxo-7-[2-[4-(trifluoromethyl)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile, (232C)

Compound 232B (0.075 g, 0.14 mmol) was dissolved in acetone (2.0 mL) and N-methylmorpholine-N-oxide (50% aq. solution, 0.025 mL, 0.21 mmol) was added. OsO_4 (4% aq. solution, 0.007 mmol) was then added. After 3 h at 25° C., the reaction was complete and sodium sulfite (0.25 g) was added with vigorous stirring. After 15 minutes, brine (5 mL) was added and the solution extracted with EtOAc (3×10 mL). The organics were dried over anhydrous sodium sulfate and then concentrated in vacuo. The crude diol was purified by preparative TLC on silica gel, eluting with 10% acetone in chloroform to give 0.038 g (48%) of compound 232C as a yellow solid. HPLC: 98% at 3.747 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 593.08 $[M+Na]^+$.

EXAMPLE 233

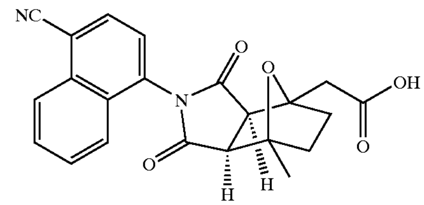
(3aα,4β,5β,5aβ,8aβ,8bα)-4-(Decahydro-5-hydroxy-4-methyl-1,3-dioxo-4,8a-epoxy-2H-furo[3,2-e]isoindol-2-yl)-1-naphthalenecarbonitrile, (233)



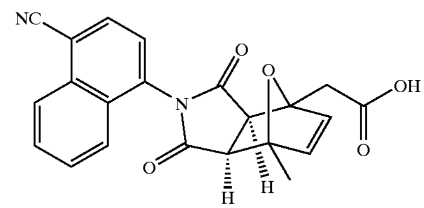
To a solution of triphenylphosphine (0.072 g, 0.28 mmol) in THF (3.0 mL) was added DBAD (0.063 g, 0.28 mmol). After 10 min, 4-cyanophenol (0.033 g, 0.28 mmol) was added. After 10 additional minutes, compound 231C (0.075 g, 0.18 mmol) was added. After 3 h at 25° C., the reaction was concentrated in vacuo and purified by preparative TLC on silica gel, eluting with 15% acetone in chloroform to give 0.068 g (95%) of compound 233 as a white solid. HPLC: 95% at 2.430 and 2.560 min (atropisomers, retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 391.09 $[M+H]^+$.

EXAMPLE 234

(3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindole-4-acetic Acid, (234B)



A. (3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl)-1,2,3,3a,7,7a-hexahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindole-4-acetic Acid (234A)



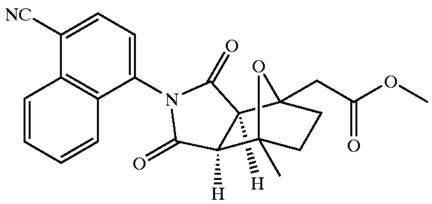
5-Methyl-2-furanacetic acid (0.500 g, 3.57 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-1-yl)-1-naphthalenecarbonitrile (0.899 g, 3.57 mmol) were dissolved in benzene (3.0 mL) and heated at 60° C. for 2 h and then cooled to 25° C. After 12 h, a white solid precipitated out of solution which was collected and rinsed with diethyl ether to yield 1.20 g (87%) of compound 234A as a light yellow solid. NMR analysis showed only one diastereomer. HPLC: 86% at 2.767 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 389.45 $[M+H]^+$.

B. (3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindole-4-acetic Acid, (234B)

Compound 234A (1.10 g, 2.82 mmol) was dissolved in EtOH/EtOAc (1:1, 50 mL) and 10% Pd/C (0.4 g, cat.) was added. H_2 was introduced via a balloon. After 5 h at 25° C., the reaction was filtered through Celite rinsing with EtOAc and concentrated in vacuo to yield 1.00 g (91%) of compound 234B as a yellow solid. HPLC: 80% at 2.84 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 391.1 $[M+H]^+$.

EXAMPLE 235

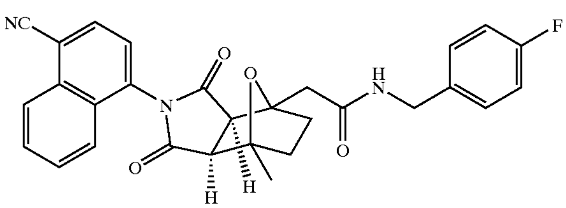
(3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl) octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindole-4-acetic Acid, Methyl Ester, (235)



Compound 234B (0.050 g, 0.13 mmol) was dissolved in acetonitrile (2.0 mL), then DCC (0.025 g, 0.13 mmol) was added followed by HOAc (0.018 g, 0.13 mmol). 4-Fluorobenzyl alcohol (0.014 mL, 0.13 mmol) was then added and the reaction was stirred for 3 h. The reaction mixture was concentrated in vacuo and purified by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm, 10–90% aqueous methanol over 15 min containing 0.1% TFA, 20 mL/min, monitoring at 220 nm). Purification yielded 0.040 g (82%) of compound 235 as a white solid, rather than the expected benzyl ester. None of the anticipated benzyl ester was observed by NMR or LC-MS. HPLC: 100% at 3.033 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 405.51 $[M+H]^+$.

EXAMPLE 236

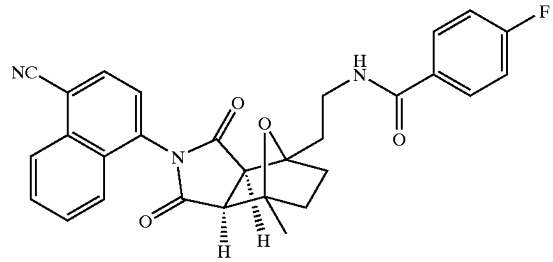
(3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl)-N-[(4-fluorophenyl)methyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindole-4-acetamide, (236)



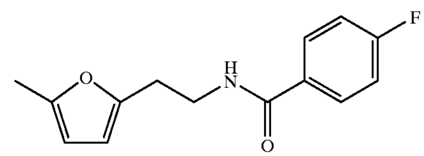
Compound 234B (0.10 g, 0.27 mmol) was dissolved in acetonitrile (4.0 mL). HOAc (0.035 g, 0.27 mmol) and DCC (0.049 g, 0.27 mmol) were then added followed by 4-fluorobenzylamine (0.030 mL, 0.27 mmol). After 4 h at 25° C., the reaction was concentrated in vacuo and purified by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm, 10–90% aqueous methanol over 15 minutes containing 0.1%. TFA, 20 mL/min, monitoring at 220 nm) to yield 0.085 g (67%) of compound 236 as a white solid. HPLC: 100% at 3.277 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 498.43 $[M+H]^+$.

EXAMPLE 237

(3aα,4β,7β,7aα)-N-[2-[2-(4-Cyano-1-naphthalenyl) octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethyl]-4-fluorobenzamide, (237B)



A. 4-Fluoro-N-[2-(5-methyl-2-furanyl)ethyl] benzamide (237A)



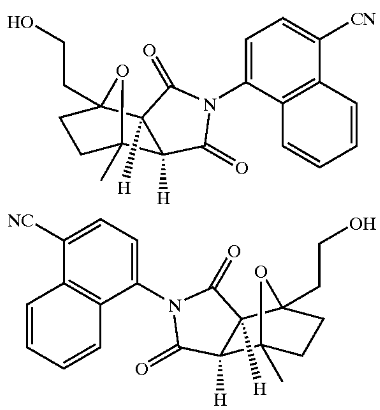
4-Fluorophenylacetyl chloride (0.290 mL, 2.44 mmol) was added dropwise to a solution of β-(5-methyl-2-furanyl) ethanamine (300 mg, 2.44 mmol, made according to the procedure of Yur'ev et al. *J. Gen. Chem. USSR* (*Engl. Transl.*) 33, 3444–8 (1963)) in THF (2.5 mL) at rt, followed by the dropwise addition of Et_3N (0.340 mL, 2.44 mmol). Once the starting material was consumed, as was evident by HPLC, the reaction was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with a gradient of 0–50% EtOAc/hexane gave 523 mg (95%) of compound 237A as a white solid. HPLC: 99% at 2.84 min (retention time) (Phenomenex-prime S5-C_{18} column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 248.15 $[M+H]^+$.

B. (3aα,4β,7β,7aα)-N-[2-[2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethyl]4-fluorobenzamide, (237B)

A solution of compound 237A (221 mg, 0.896 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-1-yl)-1-naphthalenecarbonitrile (222 mg, 0.896 mmol) in benzene (4 mL) was heated at 60° C. overnight. The reaction mixture was concentrated under reduced pressure and dissolved in EtOAc (30 mL). 10% Pd/C (50 mg) was added and the mixture was stirred under a hydrogen balloon overnight. The reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 25%–75% EtOAc/hexane (gradient) gave 160 mg (36%) of compound 237B as an off-white solid. HPLC: 97% at 3.13 & 3.23 min (atropisomers, retention time) (Phenomenex-prime S5-C18 column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 498.11 $[M+H]^+$.

EXAMPLE 238

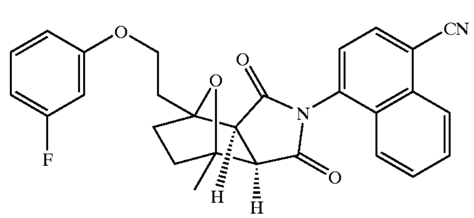
[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile & [3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile. (238i & 238ii)



Racemic compound 223B was separated into its enantiomers by normal phase preparative chiral HPLC (CHIRALPAK AD 5×50 cm column; eluting with 20% MeOH/EtOH (1:1) in heptane (isocratic) at 50 mL/min, monitoring at 220 nm) to give the faster eluting compound 238i (Chiral HPLC: 13.54 min; CHIRALPAK AD 4.6×250 mm column; eluting with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min) and the slower eluting compound 238ii (Chiral HPLC: 14.99 min; CHIRALPAK AD 4.6×250 mm column; eluting with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min). The absolute conformation for compounds 238i & 238ii was not established. For simplicity in nomenclature, compound 238i is designated herein as having an "R" configuration and compound 238ii as having an "S" configuration. Enantiomerically pure products derived from compound 238i are designated herein as having a "R" configuration and enantiomerically pure products derived from compound 238ii are designated herein as having an "S" configuration.

EXAMPLE 239

[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(3-Fluorophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile & [3aS-(-(3aα,4β,7β,7aα)]-4-[4-[2-(3-Fluorophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (239i & 239ii)



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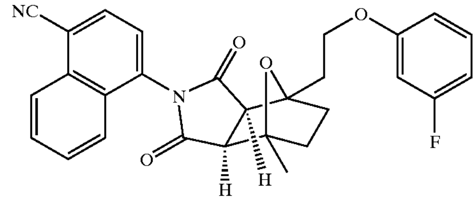
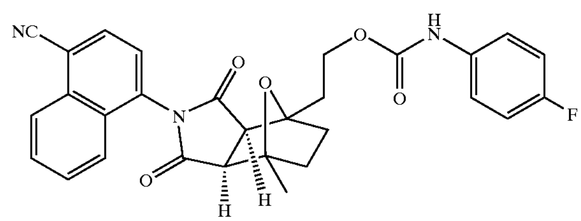


To a solution of triphenylphosphine (0.052 g, 0.20 mmol) in THF (2.0 mL) was added DBAD (0.046 g, 0.20 mmol). After 10 min, 3-fluorophenol (0.018 mL, 0.20 mmol) was added. After 10 additional minutes, compound 238i (0.050 g, 0.13 mmol) was added. After 3 h at 25° C., the reaction was concentrated in vacuo and purified by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm, 10–90% aqueous methanol over 15 minutes containing 0.2% TFA, 20 mL/min, monitoring at 220 nm) to give 0.031 g (33%) of compound 239i as a white solid. This process was repeated with compound 238ii to yield compound 239ii. Compound 239i: HPLC: 100% at 3.80 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 471.65 $[M+H]^+$, $[\alpha]_D^{25}$=−47.371 (c=4.412 mg/cc, CH_2Cl_2). Compound 239ii: HPLC: 100% at 3.80 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 471.65 $[M+H]^+$, $[\alpha]_D^{25}$=+24.3 (c=4.165 mg/cc, CH_2Cl_2).

EXAMPLE 240

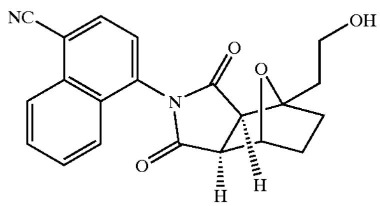
(4-Fluorophenyl)carbamic Acid, 2-[(3aα,4β,7β,7aα)-2-(4-cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-y]ethyl Ester, (240)



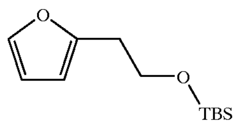
Compound 223B (0.100 g, 0.279 mmol) was dissolved in dichloroethane (3.0 mL) and 4-fluorophenylisocyanate (0.048 mL, 0.42 mmol) was added followed by heating to 60° C. After 2 h, the reaction was cooled to 25° C. and diluted with methylene chloride. The mixture was washed once with sat. aq. sodium bicarbonate (20 mL) and then the organics were dried over anhydrous sodium sulfate. The crude material was purified by flash chromatography on silica gel eluting with 15% acetone in chloroform to give 0.098 g (68%) of compound 240 as a yellow foam. HPLC: 98% at 3.320 & 3.457 min (atropisomers, retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 514.13 $[M+H]^+$.

EXAMPLE 241

(3aα,4β,7β,7aα)-4-[Octahydro-4-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (241D)

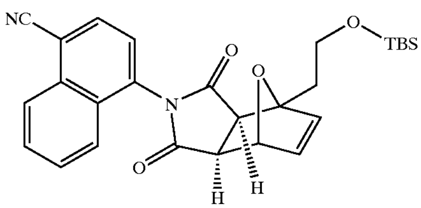


A. 2-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]furan (241A)



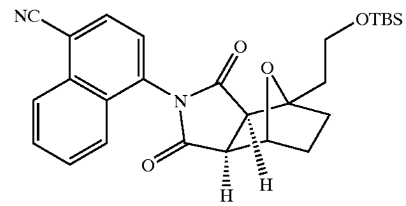
2-(2-Hydroxyethyl)furan (1.00 g, 8.93 mmol, Example 255A) was dissolved in DMF at 25° C. and imidazole (0.790 g, 11.6 mmol) was added. TBSCl (1.35 g, 8.93 mmol) was then added in portions over 5 minutes. After 2 h, the reaction was poured into diethyl ether (300 mL) and washed sequentially with water (1×100 mL), 1 N HCl (1×100 mL), and brine (1×100 mL). The combined organics were then dried over magnesium sulfate and concentrated in vacuo. Compound 241A was isolated as a clear oil (1.77 g) and was taken on without purification. HPLC: 100% at 4.233 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

B. (3aα,4β,7β,7aα)-4-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-1,3,3a,4,7,7a-hexahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (241B)



4-(2,5-Dihydro-2,5-dioxo-1H-1-yl)-1-naphthalenecarbonitrile (0.721 g, 3.40 mmol) was suspended in benzene (5.0 mL) in a sealed tube and compound 241A (1.00 g, 4.42 mmol) was added. The reaction was heated at 60° C. for 16 h and then cooled to 25° C. The benzene was removed in vacuo to give a yellow solid. The crude material was purified by flash chromatography on silica gel eluting with 1–5% acetone in chloroform to give 1.37 g (85%) of compound 241B as a yellow solid. NMR experiments confirmed the exo isomer assignment. HPLC: 100% at 4.030 & 4.110 min (atropsiomers, retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

C. (3aα,4β,7β,7aα)-4-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (241C)



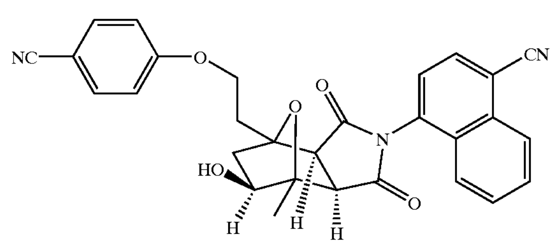
Compound 241B (0.500 g, 1.14 mmol) was dissolved in ethyl acetate (40 mL) and 10% Pd/C (0.200 g) was added. Hydrogen was then introduced via a balloon. After 4 h, the reaction was filtered through Celite, rinsed with ethyl acetate and concentrated in vacuo to yield a pale yellow solid, which was purified by flash chromatography on silica gel eluting with acetone/chloroform (0%–1.5%–3% acetone) to give 0.450 g (83%) of compound 241C as a yellow foam.

D. (3aα,4β,7β,7aα)-4-[Octahydro-4-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (241D)

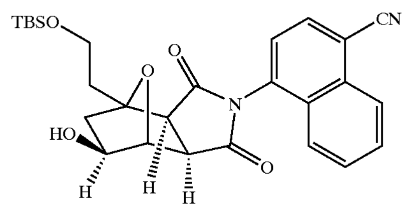
Compound 241C (0.283 g, 0.594 mmol) was dissolved in a solution of 2% conc. HCl in absolute ethanol (10 mL). After 1 h, the reaction was quenched with sat. aq. sodium bicarbonate and extracted with methylene chloride (4×20 mL). The combined organics were dried over sodium sulfate and concentrated in vacuo to give 0.211 g (98%) of compound 241D as a white solid. HPLC: 100% at 2.14 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 363.45 $[M+H]^+$.

EXAMPLE 242

(3aα,4β,6β,7β,7aα)-4-[4-[2-(4-Cyanophenoxy)ethyl]octahydro-6-hydroxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (242C)

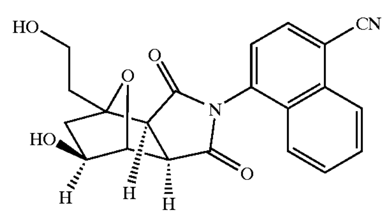


A. (3aα,4β,6β,7β,7aα)-4-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-6-hydroxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (242A)



Compound 241B (1.00 g, 2.28 mmol) and Wilkinson's catalyst (0.105 g, 0.114 mmol) were stirred rapidly under vacuum at 25° C. for 1 h and then purged with N_2. THF (30 mL) was then added followed by catecholborane (0.487 mL, 4.57 mmol) after the olefin was completely dissolved. After 1 h, the reaction was cooled to 0° C. and a pH 7.2 phosphate buffer (33 mL) was added followed by EtOH (13 mL) and H_2O_2 (30% aq. soln, 3.0 g). After 3 h at 0° C. the reaction was complete by LC and the mixture was extracted with methylene chloride (3×50 mL). The combined organics were washed with a 1:1 mixture of 10% sodium sulfite/1 N NaOH (50 mL) and once with brine (50 mL). All aqueous phases were combined and extracted with methylene chloride (50 mL) and the organic phase combined with the previous extractions. All the organics were then dried over anhydrous sodium sulfate and then concentrated in vacuo. The crude material was purified by flash chromatography on silica gel eluting with 10–20% acetone in chloroform to give 0.634 g of compound 242A as a white foam. HPLC: 96% at 3.797 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 493.13 $[M+H]^+$.

B. (3aα,4β,6β,7β,7aα)-4-[Octahydro-6-hydroxy-4-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (242B)



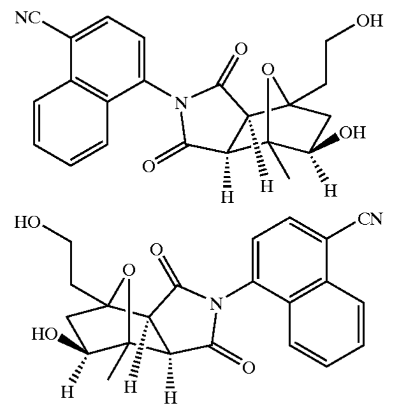
Compound 242A (0.400 g, 0.813 mmol) was dissolved in a solution of 2% 12 N HCl in absolute ethanol (10 mL). After 1 h, the reaction was quenched with sat. aq. sodium bicarbonate and extracted with EtOAc (4×20 mL). The combined organics were dried over sodium sulfate and concentrated in vacuo to give 0.305 g of compound 242B as a white solid. HPLC: 90% at 2.043 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 379.09 $[M+H]^+$.

C. (3aα,4β,5β,7β,7aα)-4-[4-[2-(4-Cyanophenoxy)ethyl]octahydro-6-hydroxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (242C)

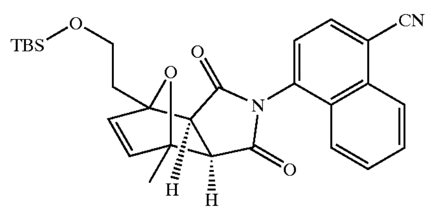
To a solution of triphenylphosphine (0.054 g, 0.207 mmol) in THF (2.0 mL) was added DBAD (0.048 g, 0.207 mmol). After 10 min, 4-cyanophenol (0.025 g, 0.207 mmol) was added. After 10 additional minutes, compound 242B (0.050 g, 0.138 mmol) was added. After 3 h at 25° C., the reaction was concentrated in vacuo and purified by preparative TLC on silica eluting with 25% acetone/chloroform to give 0.056 g of compound 242C as a white solid. HPLC: 90% at 2.987 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 480.10 $[M+H]^+$.

EXAMPLE 243

[3aS-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-7-(2-hydroxyethyl)-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile & [3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-7-(2-hydroxyethyl)-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (243Di & 243Dii)



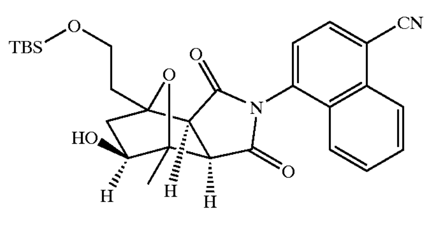
A. (3aα,4β,7β,7aα)-4-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-1,3,3a,4,7,7a-hexahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (243A)



4-(2,5-Dihydro-2,5-dioxo-1H-1-yl)-1-naphthalenecarbonitrile (18.3 g, 68.7 mmol) was added to a solution of compound 204A (26.6 g, 110.6 mmol) in benzene (75 mL) and heated at 60° C. overnight. After cooling to rt, the reaction mixture was concentrated under reduced pressure. The residue was treated with MeOH (250 mL) with stirring at 0° C. for 10 min. The resulting solid was filtered, washed with cold MeOH (2×10 mL) and dried to give 26.7 g (79.5%) of compound 243A as a yellow solid. HPLC analysis of the above solid revealed it to be 95% pure (HPLC conditions: 95% at 2.48 min (retention time) (Phenomenex-prime S5-C18 column, 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm)). The filtrate was then concentrated under

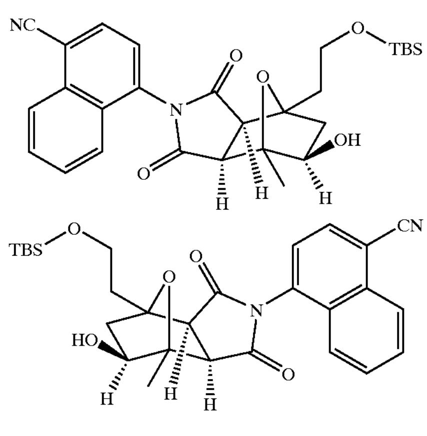
reduced pressure and the resulting solid was chromatographed, eluting with 3% acetone/$CHCl_3$, to give an additional 4.36 g of compound 243A (13%), giving a total final yield of 92.5%.

B. (3aα,4β,5β,7β,7aα)-4-[7-[2-[[(,1-Dimethylethyl) dimethylsilyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (243B)



A mixture of 243A (10 g, 20.46 mmol) and $RhCl(PPh_3)_3$ (0.947 mg, 1.02 mmol) was evacuated and filled with argon (3×). THF (200 mL) was added and once all particulates had dissolved, catecholborane (4.4 mL, 40.93 mmol) was slowly added dropwise. When the formation of product ceased, as was determined by HPLC, the reaction mixture was cooled to 0° C. and quenched with phosphate buffer (330 mL, pH 7.2) then EtOH (130 mL) and H_2O_2 (300 mL, 30% aq. sol) were added. Once boronate was consumed, the mixture was extracted with CH_2Cl_2 (3×) and the combined organic layers were washed with 1 N NaOH, 10% aq. $NaHSO_3$ (1:1, 1×) and brine (1×). The combined washes was extracted with CH_2Cl_2 (1×) and the combined organic layers were dried over Na_2SO_4. Purification by flash chromatography on silica gel eluting with 10% to 30% acetone/$CHCl_3$ gradient over 25 min gave 7.1 g (68%) of 243B as a light yellow solid. HPLC conditions: 98% at 3.82 min (retention time) (Phenomenex-prime S5-C18 column 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm).

C. [3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile & [3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[[(1,1-Dimethylethyl) dimethylsilyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (243Ci & 243Cii)



The racemic compound 243B was separated into the individual enantiomers by chiral normal phase liquid chromatography. A Chiralpak OD column (50×500 mm) was used, eluting with 13% EtOH/hexanes over 99 min at 50 mL/min detecting at 220 nm. The faster eluting isomer compound 243Ci had a retention time=45 min and the slower eluting isomer compound 243Cii had a retention time=66 min.

D. [3aS-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-7-(2-hydroxyethyl)-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile & [3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-7-(2-hydroxyethyl)-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (243Di & 243Dii)

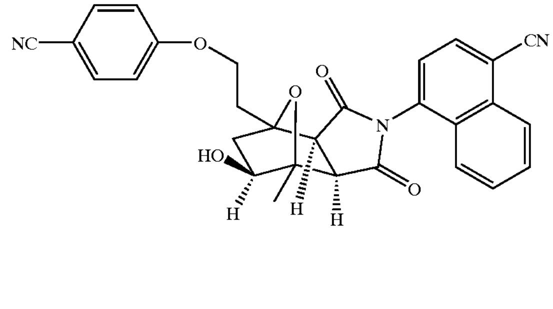
Compound 243Ci (0.84 g, 2.14 mmol) was dissolved in 2% 12 N HCl/EtOH (20 mL), stirred for 5 minutes and concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 5–10% MeOH/CH_2Cl_2 gave 0.57 g (88%) of 243Di. Compound 243Di which came from the faster eluting isomer (243Ci) was found to be 99.7% ee by analytical normal phase chiral chromatography. HPLC conditions: 99.7% at 2.17 min (retention time) (Chiralcel OJ 44.6×250 mm, 10 micron, 40° C., isocratic 80% Heptane 20% EtOH/MeOH (1:1), 1.0 mL/min., detection at 288 nm).

Compound 243Cii (0.86 g, 2.19 mmol) was dissolved in 2% 12 N HCl/EtOH (20 mL), stirred for 5 minutes and concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 5–10% MeOH/CH_2Cl_2 gave 0.60 g (90%) of 243Dii. Compound 243Dii which came from the slower eluting isomer (243Cii) was found to have 87.1% ee by analytical chiral phase chiral chromatography. HPLC conditions: 87.1% at 18.4 min (retention time) (Chiralcel OJ 44.6×250 mm, 10 micron, 40° C., isocratic 80% heptane 20% EtOH/MeOH (1:1), 1.0 mL/min., detection at 288 nm).

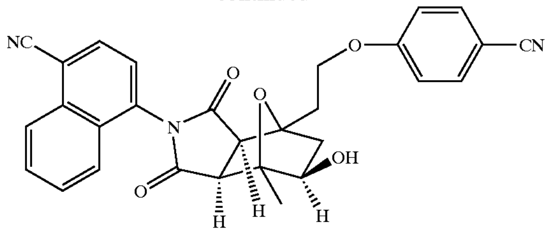
The absolute conformation for compounds 243Di & 243Dii was not determined. For simplicity in nomenclature, compound 243Di is designated herein as having an “S” configuration and compound 243Dii as having an “R” configuration. Enantiomerically pure products derived from compound 243Di are designated herein as having an “S” configuration and enantiomerically pure products derived from compound 243Dii are designated herein as having an “R” configuration.

EXAMPLE 244

[3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(4-Cyanophenoxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile & [3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(4-Cyanophenoxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (244i & 244ii)



-continued

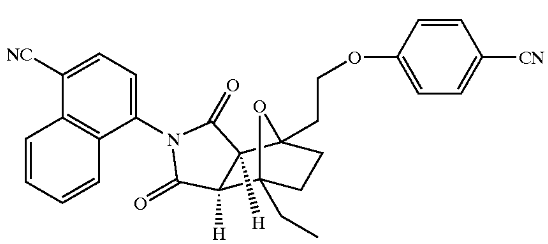


DBAD (26 mg, 0.115 mmol) was added to a solution of PPh_3 (30 mg, 0.115 mmol) in THF (0.65 mL). After stirring for 10 min, 4-cyanophenol (13.6 mg, 0.115 mmol) was added and the reaction mixture was stirred for a further 5 min. Compound 243Di (30 mg, 0.076 mmol) was added and the mixture was stirred at rt for 1 h. The reaction was concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 30% acetone/70% $CHCl_3$ gave 23.1 mg (0.047 mmol, 61.7%) of compound 244i. HPLC conditions: 95% at 3.06 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm). MS (ES): m/z 494.09 $[M+H]^+$. $[\alpha]_D$=53.30°, C=4.5 mg/cc in THF, @ 589 nm).

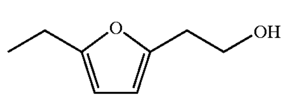
DBAD (26 mg, 0.115 mmol) was added to a solution of PPh_3 (30 mg, 0.115 mmol) in THF (0.65 mL). After stirring for 10 min, 4-cyanophenol (13.6 mg, 0.115 mmol) was added and the reaction mixture was stirred for a further 5 min. Compound 243Dii (30 mg, 0.076 mmol) was added and the mixture was stirred at rt for 1 h. The reaction was concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 30% acetone/70% $CHCl_3$ gave 20.3 mg (0.041 mmol, 54.2%) of compound 244ii. HPLC conditions: 90% at 3.07 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm). MS (ES): m/z 494.09 $[M+H]^+$. $[\alpha]_D$=−42.870°, C=6.6 mg/cc in THF, @ 589 nm).

EXAMPLE 245

(3aα,4β,7β,7aα)-4-[4-[2-(4-Cyanophenoxy)ethyl]-7-ethyloctahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (245D)

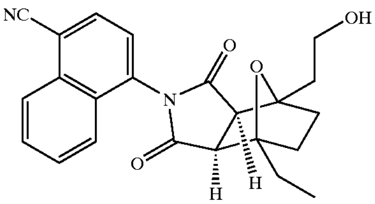


A. 2-Ethyl-5-(2-hydroxyethyl)furan (245A)



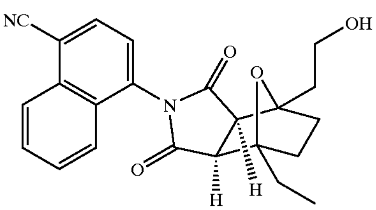
n-BuLi (2.5 M in hexane, 4.4 mL, 11 mmol) was added to a solution of 2-ethylfuran (1.05 mL, 10 mmol) in THF (10 mL) at −25° C. The solution was warmed to rt and stirred for 3 h. Ethylene oxide (0.75 mL) was added at −78° C. The reaction was stirred for 0.5 h at −15° C. and overnight at rt. Aqueous sat. NH_4Cl was added and the mixture was extracted with ether (3×). The combined extracts were washed with water (1×) and brine (1×) and dried over Na_2SO_4. Purification by flash chromatography on silica gel eluting with 30% EtOAc/70% hexane gave 1.12 g. (8.02 mmol, 80.2%) of compound 245A as a yellow oil.

B. (3aα,4β,7β,7aα)-4-[4-Ethyl-1,3,3a,4,7,7a-hexahydro-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (245B)



A solution of compound 245A (280 mg, 2.00 mmol) and the 4-(2,5-dihydro-2,5-dioxo-1H-1-yl)-1-naphthalenecarbonitrile (496 mg, 2.00 mmol) in benzene (2 mL) was stirred at 60° C. for 2 h. The reaction mixture was concentrated under reduced pressure. The yellow solid, compound 245B, was used directly in the next step.

C. (3aα,4β,7β,7aα)-4-[4-Ethyloctahydro-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (245C)



A mixture of compound 245B (764 mg, 1.97 mmol) and 10% Pd/C (115 mg, cat.) in EtOAc (36 mL) was stirred under a hydrogen atmosphere at rt for 2 h. The reaction mixture was filtered through Celite and concentrated under reduced pressure to give 779 mg of crude compound 245C. Purification of this crude product by flash chromatography on silica gel eluting with 70% EtOAc/30% hexane gave 235 mg (0.6 mmol, 30.1%) of compound 245C. HPLC conditions: 99% at 2.84 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm). MS (ES): m/z 391.12 $[M+H]^+$.

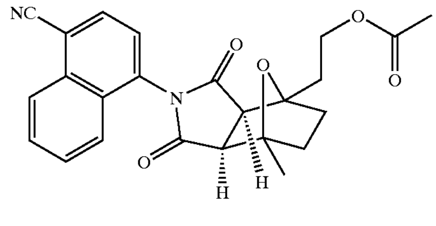
D. (3aα,4β,7β,7aα)-4-[4-[2-(4-Cyanophenoxy)ethyl]-7-ethyloctahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (245D)

DBAD (44.2 mg, 0.192 mmol) was added to a solution of PPh_3 (50.4 mg, 0.192 mmol) in THF (1 mL). After stirring for 10 min, 4-cyanophenol (23 mg, 0.192 mmol) was added and the reaction mixture was stirred for an additional 5 min. Compound 245C (50 mg, 0.128 mmol) was added and the mixture was stirred at rt for 2 h. The reaction was concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 40% EtOAc/60% hexane

gave 43 mg (0.087 mmol, 68.4%) of compound 245D as a white solid. HPLC conditions: 99% at 3.65 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm). MS (ES): m/z 492.16 $[M+H]^+$.

EXAMPLE 246

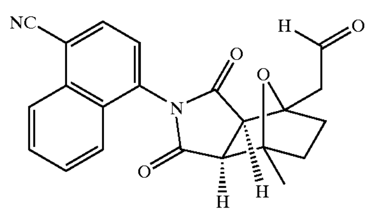
(3aα,4β,7β,7aα)-4-[2-(Acetyloxy)ethyl]-2-(4-cyano-1-naphthalenyl)hexahydro-7-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione, (246)



Compound 223B (0.100 g, 0.279 mmol) was dissolved in methylene chloride (3.0 mL) at 25° C. and pyridine (0.071 mL, 0.837 mmol) and 4-DMAP (1.0 mg) were added. Acetic anhydride (0.053 mL, 0.559 mmol) was then added and the reaction was stirred for 20 h at 25° C. After 20 h, sat. aq. sodium bicarbonate was added and the reaction was stirred for 30 min. The mixture was then extracted with methylene chloride (2×20 mL). The organics were then washed once with 1 N HCl (10 mL) and then dried over anhydrous sodium sulfate. After concentration in vacuo, the crude material was purified by preparative TLC on silica eluting with 12% acetone in chloroform to give 0.073 g of compound 246 as a yellow foam. HPLC: 95% at 2.837 and 3.027 min (atropisomers, retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 441.10 $[M+Na]^+$.

EXAMPLE 247

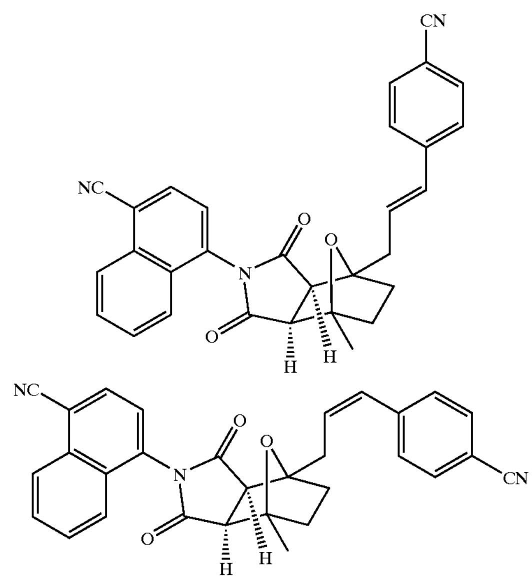
(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-(2-oxoethyl)-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (247)



Oxalyl chloride (2.0 M soln, 1.73 mL, 3.5 mmol) was added to dry methylene chloride (10 mL) and cooled to −78° C. DMSO (0.283 mL, 3.99 mmol) was then added dropwise with the evolution of gas. After 15 min, compound 223B (1.00 g, 2.66 mmol) was then added in methylene chloride (10 mL). After 15 min, TEA (1.10 mL, 7.98 mmol) was added and the reaction was slowly warmed to 25° C. Water (30 mL) was then added and the mixture was diluted with methylene chloride (100 mL). The organics were then washed once with 1 N HCl (30 mL), once with water (30 mL) and once with brine (30 mL) and then dried over anhydrous sodium sulfate. The crude product was isolated by concentration in vacuo to yield compound 247 as an orange foam. Crude compound 247 was taken on directly to the next reaction. HPLC: 100% at 2.70 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 483.65 $[M+H]^+$.

EXAMPLE 248

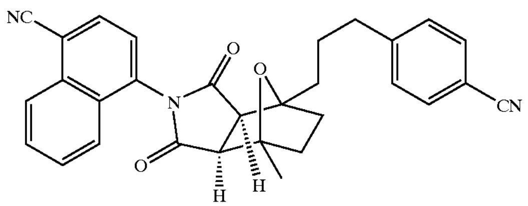
[3aα,4β(E),7β,7aα]-4-[4-[3-(4-Cyanophenyl)-2-propenyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile & [3aα,4β(Z),7β,7aα]-4-[4-[3-(4-Cyanophenyl)-2-propenyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (248i & 248ii)



(4-cyanobenzyl)-triphenylphosphonium chloride (0.072 g, 0.174 mmol) was suspended in THF (2.0 mL) and cooled to 0° C. n-BuLi (1.6 M soln, 0.092 mL, 0.147 mmol) was then added dropwise resulting in a homogenous solution. The solution warmed to 25° C. for 15 min and then cooled to 0° C. Compound 247 (0.050 g, 0.134 mmol) was then added in THF. After 1 h, the reaction was quenched with sat. aq. ammonium chloride and then extracted with methylene chloride (3×20 mL). The combined organics were dried over anhydrous sodium sulfate and then concentrated in vacuo. The crude material was purified by preparative TLC eluting with 5% acetone in chloroform to give 0.010 g of a mixture of compounds 248i & 248ii as a white solid. A 1:1 mixture of E and Z olefin isomers characterized by NMR spectroscopy. HPLC: 100% at 3.517 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 474.2 $[M+H]^+$.

EXAMPLE 249

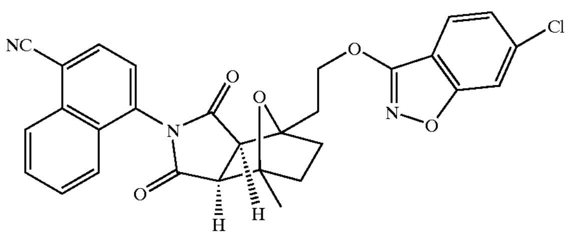
(3aα,4β,7β,7aα)-4-[4-[3-(4-Cyanophenyl)propyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (249)



The mixture of compounds 248i & 248ii (0.008 g, 0.017 mmol) was dissolved in EtOH (3.0 mL) and Pd/C (10% Pd, 0.008 g) was added. H_2 was then introduced via a balloon. After 18 h, the reaction was filtered through Celite, eluting with EtOAc, followed by concentration in vacuo. Compound 249 was isolated as a white solid (0.007 g). HPLC: 90% at 3.520 min (retention time) (YMC S5 ODS column 4.6×50 mm, 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 476.13 $[M+H]^+$.

EXAMPLE 250

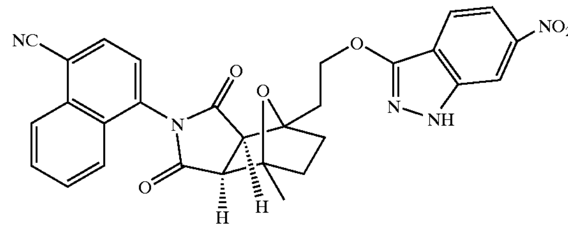
(3aα,4β,7β,7aα)-4-[4-[2-[(6-Chloro-1,2-benzisoxazol-3-yl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (250)



To a solution of PPh_3 (52 mg, 0.20 mmol) in 0.5 mL THF was added DBAD (46 mg, 0.20 mmol) as one solid portion. The resulting mixture was stirred for 10 min before 6-chloro-3-hydroxy-1,2-benzisoxazole (34 mg, 0.20 mmol) was added. Stirring was continued for 10 min before a solution of compound 223B (50 mg, 0.13 mmol) in 0.5 mL THF was introduced via canula. The resulting mixture was stirred at ambient temperature for 24 h, concentrated in vacuo and purified by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm column; eluting with 30–100% aqueous MeOH containing 0.1% TFA over 10 min at 20 mL/min) to yield a white solid. The obtained solids were dissolved in CH_2Cl_2, washed with sat. $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated in vacuo to yield 50 mg (71%) of compound 250 as a colorless oil. HPLC: 3.89 min & 4.02 min (atropisomers, retention time) (YMC S5 ODS column 4.6×50 mm Ballistic, 10–90% aqueous methanol over 4 minutes containing 0.2% H_3PO_4, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 528.4 $[M+H]^+$.

EXAMPLE 251

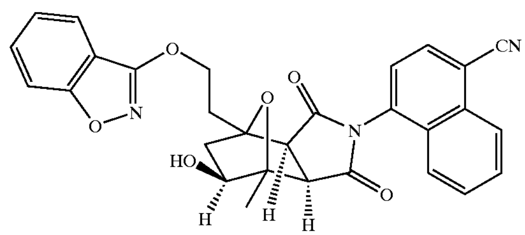
(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-7-[2-[(6-nitro-1H-indazol-3-yl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (251)



To a solution of compound 223B (50 mg, 0.13 mmol) in toluene (1 mL) was added ADDP (50 mg, 0.20 mmol), 6-nitro-3-indazolinone (36 mg, 0.20 mmol) and n-Bu_3P (50 μL, 0.2 mmol). The resulting mixture was heated at 80° C. for 24 h, concentrated in vacuo and purified by a combination of reverse phase preparative HPLC (YMC S5 ODS 20×100 mm column; eluting with 30–100% aqueous MeOH containing 0.1% TFA over 10 min at 20 mL/min) and flash chromatography (silica gel, 25% acetone in $CHCl_3$) to give 17 mg (25%) of compound 251 as a yellow solid. HPLC: 3.60 min & 3.74 min (atropisomers, retention time) (YMC S5 ODS column 4.6×50 mm Ballistic, 10–90% aqueous methanol over 4 minutes containing 0.2% H_3PO_4, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 537.6 $[M+H]^+$.

EXAMPLE 252

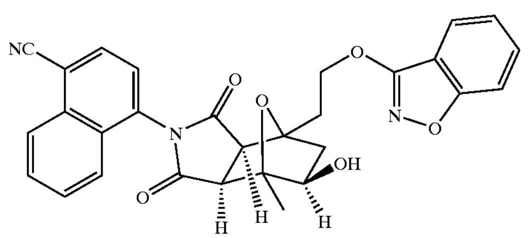
[3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(1,2-Benzisoxazol-3-yloxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (252)



PPh_3 (47 mg, 0.18 mmol), DBAD (41 mg, 0.18 mmol), 3-hydroxy-1,2-benzisoxazole (24 mg, 0.18 mmol) and compound 243Di (35 mg, 0.09 mmol) were reacted according to the procedure given for compound 250. Purification was achieved by reverse phase HPLC (YMC S5 ODS 20×100 mm column; eluting with 30–100% aqueous MeOH containing 0.1% TFA over 10 min at 20 mL/min) to yield a white solid. The obtained solids were dissolved in CH_2Cl_2, washed with sat. $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated under reduced pressure to furnish 29 mg (64%) of compound 252 as a colorless oil. HPLC: 96% at 3.29 min (atropisomers, retention time) (YMC S5 ODS column 4.6×50 mm Ballistic, 0–100% aqueous methanol over 4 minutes containing 0.2% H_3PO_4, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 510.2 $[M+H]^+$.

EXAMPLE 253

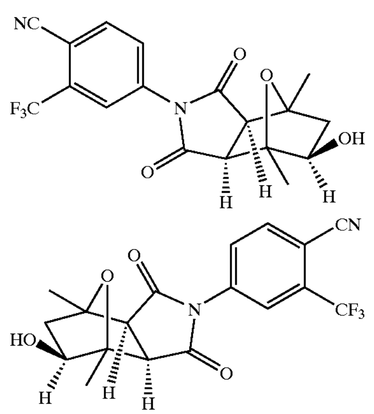
[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(1,2-Benzisoxazol-3-yloxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (253)



PPh_3 (47 mg, 0.18 mmol), DBAD (41 mg, 0.18 mmol), 3-hydroxy-1,2-benzisoxazole (24 mg, 0.18 mmol) and compound 243Dii (35 mg, 0.09 mmol) were reacted according to the procedure given for compound 250. Purification was achieved by reverse phase HPLC (YMC S5 ODS 20×100 mm column; eluting with 30–100% aqueous MeOH containing 0.1% TFA over 10 min at 20 mL/min) to yield a white solid. The obtained solids were dissolved in CH_2Cl_2, washed with sat. $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated under reduced pressure to furnish 23 mg (51%) of compound 253 as a colorless oil. HPLC: 95% at 3.29 min (atropisomers, retention time) (YMC S5 ODS column 4.6×50 mm Ballistic, 0–100% aqueous methanol over 4 minutes containing 0.2% H_3PO_4, 4 in mL/min, monitoring at 220 nm). MS (ES): m/z 510.4 $[M+H]^+$.

EXAMPLE 254

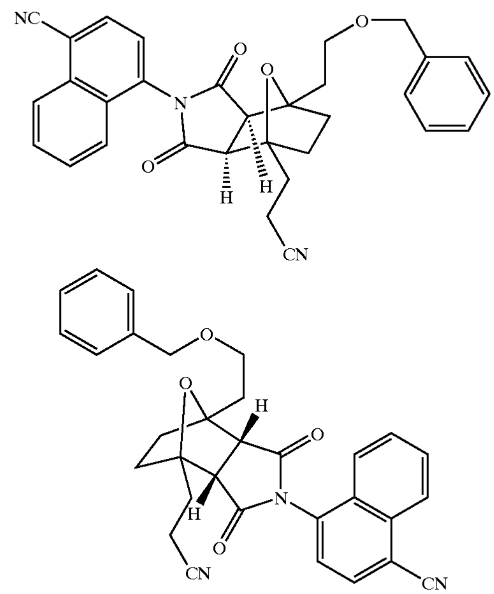
[3aR-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile & [3aS-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile, (254i & 254ii)



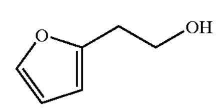
Racemic compound 221B was separated into its enantiomers by normal phase preparative chiral HPLC (CHIRALPAK AD 5×50 cm column; eluting with 20% MeOH/EtOH (1:1) in heptane (isocratic) at 50 mL/min) to give the faster eluting compound 254i (Chiral HPLC: 10.02 min; CHIRALPAK AD 4.6×250 mm column; eluting with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min) and the slower eluting 254ii (Chiral HPLC: 14.74 min; CHIRALPAK AD 4.6×250 mm column; eluting with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min). (Names of title compounds based on absolute stereochemistry determination).

EXAMPLE 255

(3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl)octahydro-1,3-dioxo-7-[2-(phenylmethoxy)ethyl]-4,7-epoxy-4H-isoindole-4-propanenitrile & (3aα,4α,7α,7aα)-2-(4-Cyano-1-naphthalenyl)octahydro-1,3-dioxo-7-[2-(phenylmethoxy)ethyl]-4,7-epoxy-4H-isoindole-4-propanenitrile, (255Hi & 255Hii)

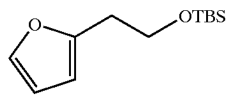


A. 2-(2-Hydroxyethyl)furan (255A)



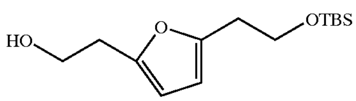
2-(2-Hydroxyethyl)furan was made in accordance with the following reference: Harmata, M, et al. *J. Org. Chem.* 60, 5077–5092 (1995). n-BuLi (2.5 M in hexane, 44 mL, 110 mmol) was added to a solution of furan (8 mL, 110 mmol) in 100 mL of THF at −78° C. The solution was stirred at 0° C. for 4 h and then ethylene oxide (7.5 mL) was added at −78° C. The reaction mixture was stirred at −15° C. for 1 h and then overnight at rt. The reaction was quenched with sat. NH_4Cl and extracted with ether (3×). The combined extracts were washed with water (1×) and brine (1×). The ether solution was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 40% EtOAc/60% hexane gave 5.4 g (48.2 mmol, 43.8%) of compound 255A as a light brown oil.

B. 2-[2-[[(1,1-Dimethlethyl)dimethylsilyl]oxy]ethyl]furan (255B)



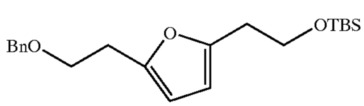
Imidazole (3.65 g, 53.6 mmol) and TBSCl (6.47 g, 42.9 mmol) were added to the solution of compound 255A (4.00 g, 35.7 mmol) in 50 mL of DMF. The mixture was stirred at rt for 2 h and then the reaction mixture was poured into ether. The ether solution was washed with water (1×), 1 N HCl (1×), water (1×) and brine (1×). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 30% CH_2Cl_2/70% hexane gave 7.4 g (32.7 mmol, 91.7%) of 255B as a colorless oil.

C. 2-[2-[[(1,1-Dimethlethyl)dimethylsilyl]oxy]ethyl]-5-(2-hydroxyethyl)furan (255C)



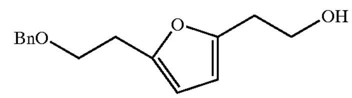
t-BuLi (1.2 M in pentane, 10 mL, 16.99 mmol) was added to a stirred solution of 255B (3.49 g, 15.44 mmol) in 13 mL of THF at −78° C. dropwise. The mixture was stirred for an additional 4 h at 0° C. Ethylene oxide (1.05 mL) was added at −78° C. to the reaction solution. The mixture was warmed to rt and stirred overnight. Aqueous sat. NH_4Cl was added and most of the THF was removed under reduced pressure. The mixture was extracted with ether (3×) and the combined organic layers were washed with water (1×) and brine (1×) and dried over Na_2SO_4. Purification by flash chromatography on silica gel eluting with 5% EtOAc/95% CH_2Cl_2 gave 2.8 g (10.4 mmol, 67%) of compound 255C as a yellow oil.

D. 2-[2-[[(1,1-Dimethlethyl)dimethylsilyl]oxy]ethyl]-5-[2-(phenylmethoxy)ethyl]furan (255D)



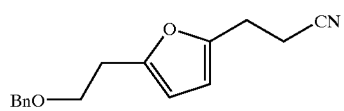
The alcohol 255C (1.00 g, 3.7 mmol) in 12 mL of THF was treated with 60% NaH (177.8 mg, 4.44 mmol), benzyl bromide (0.53 mL, 4.44 mmol) and tetrabutylammonium iodide (50 mg, 5%) for 3 h at rt. Water was added and the mixture was extracted with EtOAc (3×). The combined extracts were washed with water (1×) and brine (1×) and dried over Na_2SO_4. Purification by flash chromatography on silica gel eluting with 20% hexane/80% CH_2Cl_2 gave 1.10 g (3.05 mmol, 82.6%) of compound 255D as a yellow oil.

E. 2-(2-Hydroxyethyl)-5-[2-(phenylmethoxy)ethyl]furan (255E)



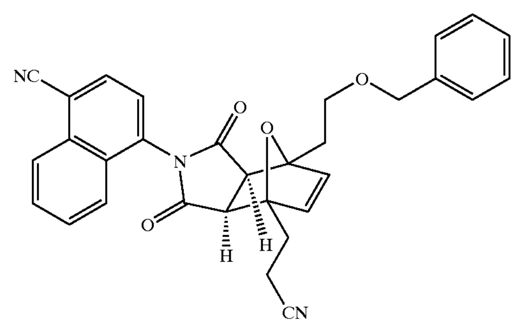
Tetrabutylammonium fluoride (1.0M in THF, 3.06 mL, 3.06 mmol) was added to the solution of compound 255D (1.1 g, 3.06 mmol) in 10 mL of THF at 0° C. The reaction mixture was stirred at rt for 10 minutes, quenched by sat. NH_4Cl and extracted with ether (3×). The combined extracts were dried over Na_2SO_4. Purification by flash chromatography on silica gel eluting with 10% EtOAc/90% CH_2Cl_2 gave 750 mg (3.05 mmol, 99.6%) of compound 255E as a light yellow oil.

F. 5-[2-(Phenylmethoxy)ethyl]furan-2-propanenitrile (255F)



DEAD (1.285 mL, 8.17 mmol) was added to a stirred solution of Ph_3P (2.14 g, 8.17 mmol) in 12 mL of dry THF at 0° C. The solution was stirred for 30 min at rt and compound 255E (670 mg, 2.72 mmol) was added. The reaction was stirred for 15 min and acetone cyanohydrin (0.745 mL, 8.17 mmol) was added at −15° C. The reaction was stirred for 30 min at −15° C., then at rt overnight. The mixture was then concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 100% CH_2Cl_2 gave 180 mg (0.705 mmol, 26%) of compound 255F as a colorless oil.

G. (3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl)-1,2,3,3a,7,7a-hexahydro-1,3-dioxo-7-[2-(phenylmethoxy)ethyl]-4,7-epoxy-4H-isoindole-4-propanenitrile (255G)



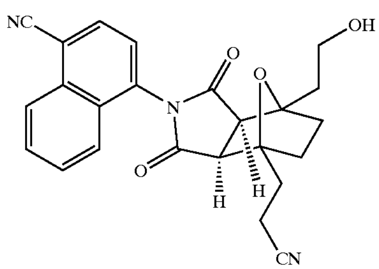
A solution of compound 255F (180 mg, 0.706 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-1-yl)-1-naphthalenecarbonitrile (263 mg, 1.06 mmol) in CH_2Cl_2 (3 mL) was stirred at rt for 3 days. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 5% EtOAc/CH_2Cl_2 gave 318 mg (0.63 mmol, 89.6%) of compound 255G as a light gray solid which was used directly in the next step.

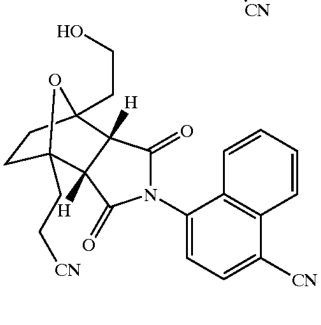
H. (3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl) octahydro-1,3-dioxo-7-[2-(phenylmethoxy)ethyl]-4,7-epoxy-4H-isoindole-4-propanenitrile & (3aα,4α,7α,7aα)-2-(4-Cyano-1-naphthalenyl)octahydro-1,3-dioxo-7-[2-(phenylmethoxy)ethyl]-4,7-epoxy-4H-isoindole-4-propanenitrile (255Hi & 255Hii)

A mixture of compound 255G (318 mg, 0.63 mmol) and 10% Pd/C (64 mg) in EtOH (10 mL) and EtOAc (5 mL) was stirred under a hydrogen atmosphere at rt overnight. The reaction mixture was filtered through Celite and concentrated under reduced pressure to give 320 mg of crude compounds 255Hi & 255Hii. Purification of 25 mg of this crude product by flash chromatography on silica gel eluting with 55% EtOAc/hexane gave 6.5 mg (0.013 mmol, 26% (based on 25 mg)) of compound 255Hi & 8.1 mg (0.016 mmol, 32.4% (based on 25 mg)) of compound 255Hii. Compound 255Hi: HPLC conditions: 98% at 3.57 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm, MS (ES): m/z 506.15 $[M+H]^+$. Compound 255Hii: HPLC conditions: 98% at 3.51 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm). MS (ES): m/z 506.15 $[M+H]^+$.

EXAMPLE 256

(3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl) octahydro-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-4H-isoindole-4-propanenitrile & (3aα,4α,7α,7aα)-2-(4-Cyano-1-naphthalenyl)octahydro-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-4H-isoindole-4-propanenitrile, (256i & 256ii)

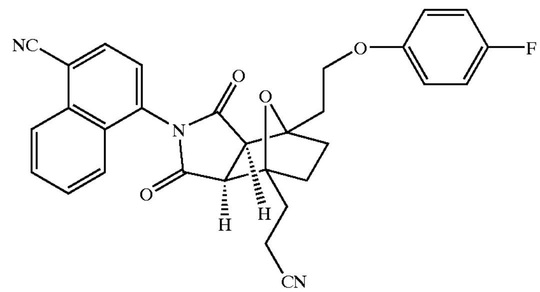




A mixture of compounds 255Hi & 255Hii (200 mg, 0.396 mmol) and $PdCl_2$ (8.4 mg, cat.) in EtOH (1 mL) and EtOAc (3 mL) was stirred under a hydrogen atmosphere (30 psi) at rt overnight. The reaction mixture was filtered through Celite and concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 5% MeOH/CH_2Cl_2 followed by a second column eluting with 100% EtOAc gave 28.9 mg (0.0696 mmol, 17.6%) of compound 256ii and 26.5 mg (0.0639 mmol, 16.1%) of compound 256i. Compound 256ii: HPLC conditions: 90% at 2.44 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm.). MS (ES): m/z 416.11 $[M+H]^+$. Compound 256i: HPLC conditions: 99% at 2.47 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm). MS (ES): m/z 416.11 $[M+H]^+$.

EXAMPLE 257

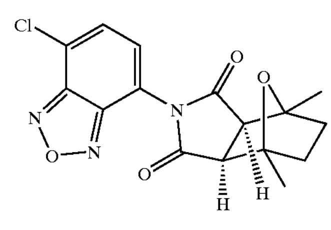
(3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl)-7-[2-(4-fluorophenoxy)ethyl]octahydro-1,3-dioxo-4,7-epoxy-4H-isoindole-4-propanenitrile. (257)



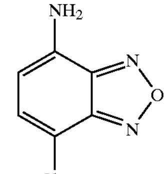
DBAD (15 mg, 0.065 mmol) was added to a solution of PPh_3 (17 mg, 0.065 mmol) in THF (0.3 mL). After stirring for 10 min, 4-fluorophenol (7.33 mg, 0.065 mmol) was added and the reaction mixture was stirred for a further 5 min. Compound 256i (18.1 mg, 0.044 mmol) was added and the mixture was stirred at rt for 3 h. The reaction was concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 60% EtOAc/30% hexane gave 5.9 mg (0.0116 mmol, 26.34%) of compound 257. HPLC conditions: 98% at 3.59 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm). MS (ES): m/z 510.14 $[M+H]^+$.

EXAMPLE 258

(3aα,4β,7β,7aα)-2-(7-Chloro-2,1,3-benzoxadiazol-4-yl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione, (258)



A. 4-Amino-7-chloro-2,1,3-benzoxadiazole (258A)



A solution of 1.0 g (5.02 mmol) of 4-chloro-7-nitrobenzofurazan in 20 mL AcOH, 10 mL EtOAc and 2 mL

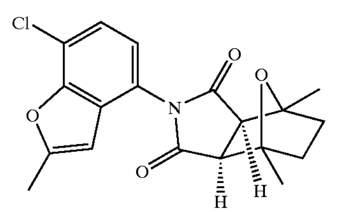
H_2O was heated at 50° C. and treated with iron powder (1.4 g, 251 mmol). The mixture was heated at 80° C. for 30 min and then allowed to cool to rt. The mixture was filtered through Celite eluting with EtOAc. The filtrate was washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, and concentrated under reduced pressure to give compound 258A (0.80 g, 94%) as a red solid.

B. (3aα,4β,7β,7aα)-2-(7-Chloro-2,1,3-benzoxadiazol-4-yl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione, (258B)

Compound 258A (42 mg, 0.25 mmol) was reacted in a sealed tube with compound 20A (73.5 mg, 0.375 mmol), $MgSO_4$ (75 mg, 0.625 mmol) and Et_3N (170 μL, 1.25 mmol) in 250 μL toluene according to the above procedure described in example 208C to give after purification by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm eluting with 30–100% aqueous methanol containing 0.1% TFA over 12 min, 20 mL/min) 23 mg (26%) of compound 258B as a yellow solid. HPLC: 97.6% at 2.87 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 4 minutes 4 mL/min, monitoring at 220 nm). MS (DCI): m/z 347.9 $[M]^+$.

EXAMPLE 259

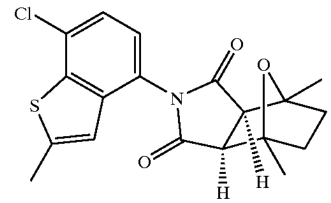
(3aα,4β,7β,7aα)-2-(7-Chloro-2-methyl-4-benzofuranyl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione, (259)



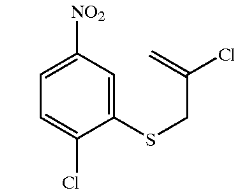
7-Chloro-2-methyl-4-benzofuranamine (38 mg, 0.25 mmol, prepared in accordance with the procedure described by Enomoto and Takemura in EP 0476697 A1) was reacted in a sealed tube with compound 20A (73.5 mg, 0.375 mmol), $MgSO_4$ (75 mg, 0.625 mmol) and Et_3N (170 μL, 1.25 mmol) in 250 μL toluene according to the procedure described in example 208C to give, after purification by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm eluting with 30–100 aqueous methanol containing 0.1% TFA over 12 min, 20 mL/min), 42 mg (47%) of compound 259 as a white solid. HPLC: 98% at 3.45 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (DCI): m/z 359.9 $[M]^+$.

EXAMPLE 260

(3aα,4β,7β,7aα)-2-(7-Chloro-2-methylbenzo[b]thiophen-4-yl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione, (260)

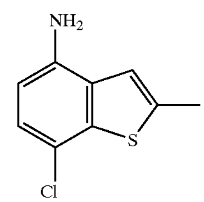


A. 1-Chloro-2-(2-chloro-allylsulfanyl)-4-nitro-benzene (260A)



A solution of 2-chloro-5-nitro-benzenethiol (1.0 g, 5.27 mmol, prepared in accordance with the procedure described by Still et al. *Synth. Comm.* 13, 1181 (1983)) in 15 mL DMF was treated with 2,3-dichloropropene (693 μL, 7.52 mmol) and K_2CO_3 (433 mg, 3.13 mmol). The mixture was heated at 80° C. for 2 h and then allowed to cool to rt. EtOAc (200 mL) and H_2O (100 mL) were added. The organic phase was washed with H_2O (2×250 mL), saturated aqueous NaCl (100 mL), dried over $MgSO_4$, and concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel eluting with 20% EtOAc in hexanes to give compound 260A (1.09 g, 89%) as an orange oil.

B. 4-Amino-7-chloro-2-methylbenzo[b]thiophene (260B)



A solution of 1.09 g (4.67 mmol) of compound 260A in 20 mL AcOH with 10 mL EtOAc and 2 mL H_2O was heated to 80° C. and treated with iron powder (1.3 g, 23.4 mmol). The mixture was heated at 80° C. for 40 min and then allowed to cool to rt. The mixture was filtered through Celite eluting with EtOAc. The filtrate was washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo. N,N-diethylaniline (10 mL) was added, and the reaction was heated at 215° C. for 6 h. After cooling to rt, 1 N aqueous. HCl (20 mL) was added, and the reaction was stirred at room temperature for 2 h. The mixture was extracted with EtOAc (3×30 mL). The organic phase was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo. The crude material was purified by flash column

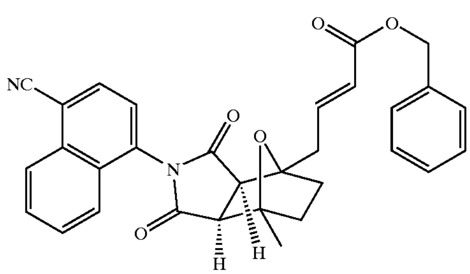
chromatography on silica gel eluting with 25% EtOAc in hexanes to give compound 260B (320 mg, 35%) as a beige solid.

C. (3aα,4β,7β,7aα)-2-(7-Chloro-2-methylbenzo[b]thiophen-4-yl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione, (260C)

Compound 260B (49 mg, 0.25 mmol) was reacted in a sealed tube with compound 20A (73.5 mg, 0.38 mmol), $MgSO_4$ (75 mg, 0.63 mmol) and Et_3N (170 μL, 1.25 mmol) in 0.250 μL toluene according to the procedure described in example 208C to give, after purification by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm eluting with 30–100% aqueous methanol over 12 min containing 0.1% TFA, 20 mL/min), 28 mg (30%) of compound 260C as a pale yellow solid. HPLC: 96% at 3.18 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (DCI): m/z 376.0. $[M]^+$.

EXAMPLE 261

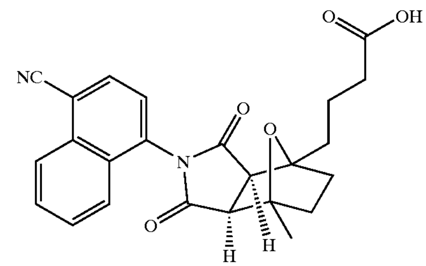
[3aα,4β(E),7β,7aα]-4-[2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]-2-butenoic Acid Phenylmethyl Ester, (261)



Compound 247 (0.500 g, 1.34 mmol) was dissolved in THF (20 mL) and benzyl(triphenylphosphoranylidene) (0.55 g, 1.34 mmol) was added. The reaction mixture was stirred at 67° C. for 2 h and then concentrated under reduced pressure. Purification by flash chromatography on SiO_2 eluting with 5% acetone/95% $CHCl_3$ gave 0.65 g of compound 261 as a yellow solid. HPLC: 99% at 3.717 min (retention time) (YMC S5. ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 507.1 $[M+H]^+$.

EXAMPLE 262

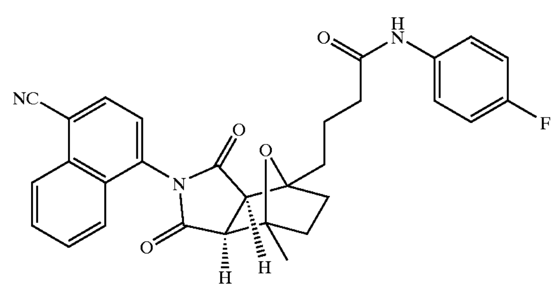
(3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindole-4-butanoic Acid, (262)



Compound 261 (0.60 g, 1.19 mmol) was dissolved in EtOH/EtOAc (5 mL/5 mL) and 10% Pd/C (0.30 g) was added. Hydrogen was then introduced via a balloon. After 8 h the reaction was filtered through Celite and then concentrated under reduced pressure to give compound 262 (0.47 g) as a white solid. HPLC: 98% at 2.81 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 419.1 $[M+H]^+$.

EXAMPLE 263

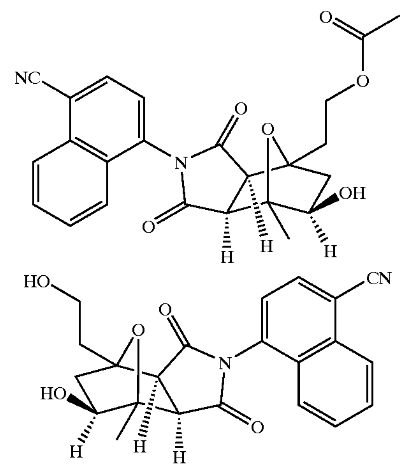
(3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl)-N-(4-fluorophenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindole-4-butanamide (263)



Compound 262 (0.030 g, 0.072 mmol) was dissolved in CH_3CN (1 mL). DCC (0.014 g, 0.072 mmol) and HOAc (0.0098 g, 0.072 mmol) were then added, followed by 4-flouroaniline (0.007 mL, 0.072 mmol). The reaction mixture was stirred under argon for 14 h and the crude material was dissolved in MeOH, purified by reverse phase preparative HPLC (YMC VP-ODS column, 20×100 mm, eluting with 20% B to 100% B in 15 minutes and hold @ 100% B for 10 minutes). Compound 263 (0.020 g) was isolated as white solid. HPLC: 100% at 3.217 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 512.1 $[M+H]^+$.

EXAMPLE 264

[3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(Acetyloxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile & [3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-7-(2-hydroxyethyl)-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (264 & 243Dii)



A racemic mixture of compounds 243Di & 243Dii (1.90 gram) were dissolved in 100 mL of anhydrous THF in a 2 L flask. Anhydrous tert-butyl-methyl ether (900 mL) and vinyl acetate (40 mL) were transferred into the flask with stirring and lipase (20 g, typeII, crude, from porcine pancreas; Sigma, Cat# L3126) was added. The reaction mixture was stirred for 21 hr at rt at which point an additional 5 grams of the lipase and 20 mL of vinyl acetate were added. The reaction was stirred at rt for an additional 19 h, stored at 4° C. without stirring for 36 h and then stirred at rt for another 22 h (until the desired % ee was apparent by chiral HPLC). To monitor the reaction, 200 uL of the mixture was withdrawn and centrifuged. The supernatant (100 uL) was dried under nitrogen and the resulting residue was dissolved in 100 uL of EtOH and subjected to HPLC analysis:

1)

Reverse phase HPLC: Column, YMC-ODS AQ 150×4.6; flow rate, 1.2 mL/min; sample size, 10 uL

solvent A,: 1 mM HCl in water; solvent B, MeCN; monitored at 300 nm

Gradient:

Time(min) 0 8 8.5 9.5 10 12

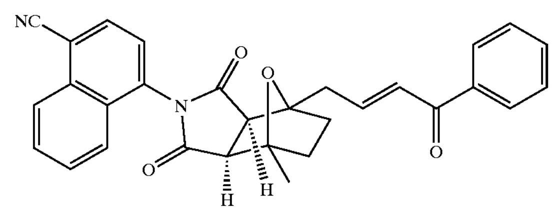
B % 30 60 85 85 30 30

2) Chiral-HPLC: Column, CHIRALCEL OJ 4.6×250 mm mobile phase, hexanes/MeOH/EtOH (8:1:1) flow rate, 1 mL/min; sample size, 20 uL monitored at both 220 and 300 nm performed at 25° C. & 40° C. (for ee % determination of reaction mixture).

The enzyme was removed by filtration and filtrate was concentrated under reduced pressure. The resulting mixture was dissolved in $CHCl_3$ and adsorbed onto silica gel (63–200 microns). These solids were applied to a VLC funnel (3 cm I.D., VLC is vacuum liquid chromatography using glass funnels having 24/40 joints at the bottom) containing a 5 cm bed height of silica gel (25–40 microns) and a step gradient was carried out. The gradient was 100% $CHCl_3$ in the first 3 fractions), followed by $CHCl_3$-1% MeOH (3 fractions), $CHCl_3$-2% MeOH (3 fractions), $CHCl_3$-3% MeOH (3 fractions), $CHCl_3$-4% MeOH (3 fractions), and finally with $CHCl_3$-5% MeOH (3 fractions). The volume of the fractions was 100 mL until reaching $CHCl_3$-3% MeOH and from that point on it was 200 mL. Compound 264 elutes in the last two fractions of 100% $CHCl_3$ and until the first fraction of $CHCl_3$-2% MeOH. Compound 243Dii elutes starting with the second fraction of $CHCl_3$-2% MeOH, and continues to the first fraction of $CHCl_3$-5% MeOH. The crude compound 243Dii contained a small amount of a colored impurity which was removed by a Sephadex column [LH-20 swollen in $CHCl_3$—MeOH (2:1), column (2.5 cm I.D. & 90 cm long) to yield 632 mg of compound 243Dii. Compound 264: HPLC conditions: 98% at 7.2 min (retention time) (method 1), chiral HPLC conditions: 29.0 min @ 25° C. (method 2). Compound 243Dii: HPLC conditions: 98% at 4.6 min (retention time) (method 1), chiral HPLC conditions: 96% ee at 25.7 min (retention time) (@25° C.) & 19.8 min (retention time) (@ 40° C.) (method 2).

EXAMPLE 265

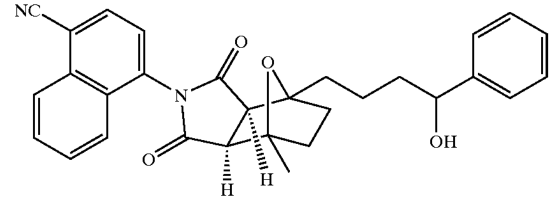
(3aα,4β,7β,7aα(E)]-4-[Octahydro-4-methyl-1,3-dioxo-7-(4-oxo-4-phenyl-2-butenyl)-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (265)



The compound 247 (0.050 g, 0.134 mmol) was dissolved in THF (1.5 mL) and (phenacylidene)triphenylphosphorane (0.051 g, 0.134 mmol) was added. The reaction mixture was stirred at 67° C. for 24 h and then cooled to 23° C. and concentrated in vacuo. The crude material was then purified by reverse phase preparative HPLC. (YMC VP-ODS column, 20×100 mm, eluting with 20% B to 100% B in 15 minutes and hold @ 100% B for 10 minutes) to give compound 265 (0.040 g) as white solid. HPLC: 100% at 3.503 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 477.1 $[M+H]^+$.

EXAMPLE 266

(3aα,4β,7β,7aα(E)]-4-[Octahydro-4-methyl-1,3-dioxo-7-(4-hydroxy-4-phenyl-2-butyl)-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (266)

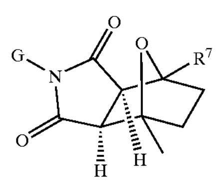


Compound 265 (0.010 g, 0.021 mmol) was dissolved in EtOH (2.0 mL) and Pd/C (10% Pd, 0.005 g) was added.

Hydrogen was then introduced via a balloon and the reaction was stirred at 25° C. for 3 h. The reaction was then filtered through Celite rinsing with EtOAc and concentrated in vacuo to give compound 266 as a tan solid (0.009 g). No purification was necessary. HPLC: 100% at 3.38 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 503.2 $[M+Na]^+$. (Where this reaction was run for 1 hour, the resulting product was compound 455.)

EXAMPLES 267 TO 378

Additional compounds of the present invention were prepared by procedures analogous to those described above. The compounds of Examples 267 to 378 have the following structure (L is a bond):



where G, R^7, the compound name, retention time, molecular mass, and the procedure employed, are set forth in Table 5. The absolute configuration for the following compounds was not determined. For simplicity in nomenclature, compound 238i is designated herein as having an "R" configuration and compound 238ii as having an "S" configuration. Enantiomerically pure products derived from compound 238i are designated herein as having an "R" configuration and enantiomerically pure products derived from compound 238ii are designated herein as having an "S" configuration.

The chromatography techniques used to determine the compound retention times of Table 5 are as follows: LCMS=YMC S5 ODS column, 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 4 minutes containing 0.1% TFA; 4 mL/min, monitoring at 220 nm. LCMS*=YMC S5 ODS column, 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 2 minutes containing 0.1% TFA; 4 mL/min, monitoring at 220 nm. LC=YMC S5 ODS column 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm. The molecular mass of the compounds listed in Table 5 were determined by MS (ES) by the formula m/z.

TABLE 5

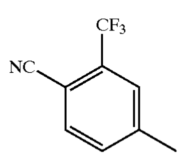
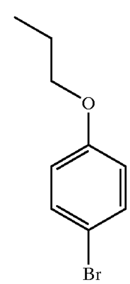
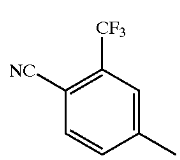
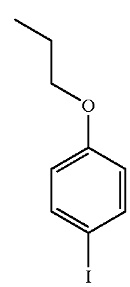
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
267			(3aα,4β,7β,7aα)-(4-[7-[2-(4-Bromophenoxy)ethyl] octahydro-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.97 LCMS 549.0 $[M + H]^+$	204, 35
268			(3aα,4β,7β,7aα)-4-[Octahydro-7-[2-(4-iodophenoxy)ethyl]-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	4.09 LCMS 597.0 $[M + H]^+$	204, 35

TABLE 5-continued

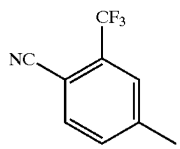
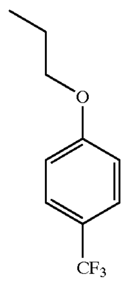
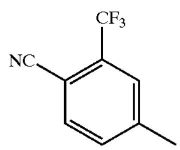
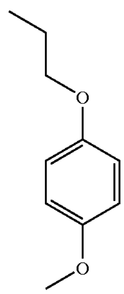
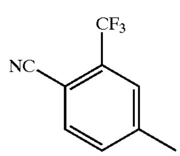
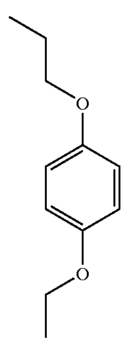
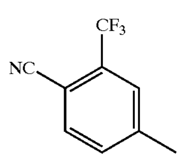
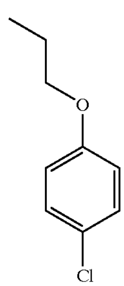
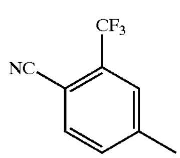
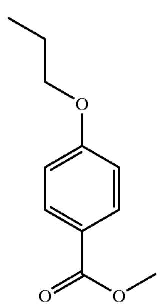
Ex. No	G	R7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
269			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[4-(trifluoromethyl)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.95 LC	204, 35
270			(3aα,4β,7β,7aα)-4-[Octahydro-7-[2-(4-methoxyphenoxy)ethyl]-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.66 LC	204, 35
271			(3aα,4β,7β,7aα)-4-[7-[2-(4-Ethoxyphenoxy)ethyl]octahydro-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.81 LC	204, 35
272			(3aα,4β,7β,7aα)-4-[7-[2-(4-Chlorophenoxy)ethyl]octahydro-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.97 LCMS 522.2 [M + H]+	204, 35
273			(3aα,4β,7β,7aα)-4-[2-[2-[4-Cyano-3-(trifluoromethyl)phenyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy]benzoic acid, methyl ester.	3.77 LCMS 529.12 [M + H]+	204, 35

TABLE 5-continued

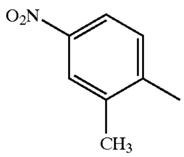
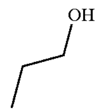
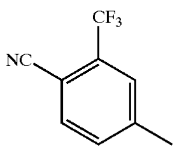
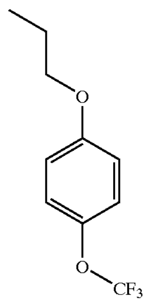
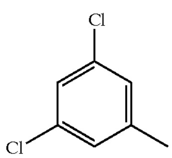
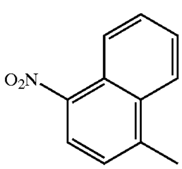
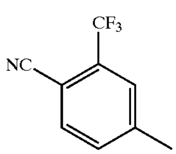
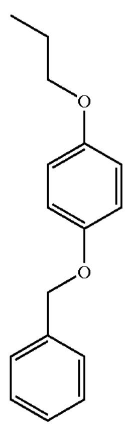
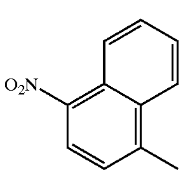
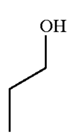
Ex. No	G	R7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
274			(3aα,4β,7β,7aα)-Hexahydro-4-(2-hydroxyethyl)-7-methyl-2-(3-methyl-4-nitrophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.44 LC	204, 35
275			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[4-(trifluoromethoxy)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.97 LC	204, 35
276		CH_3	(3aα,4β,7β,7aα)-2-(3,5-Dichlorophenyl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.31 LCMS 341.2 $[M + H]^+$	20
277		CH_3	(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.04 LCMS	20
278			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[4-(phenylmethoxy)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	4.06 LC	204, 35
279			(3aα,4β,7β,7aα)-Hexahydro-4-(2-hydroxyethyl)-7-methyl-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.607 & 2.743 rotational isomers LC	204, 35

TABLE 5-continued

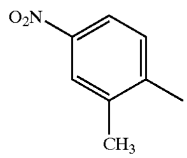
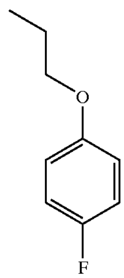
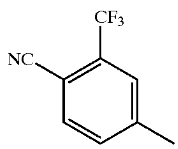
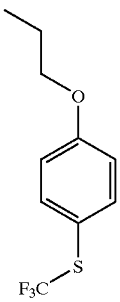
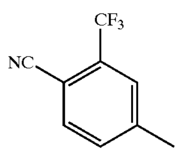
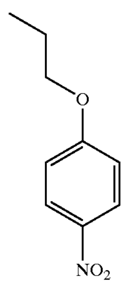
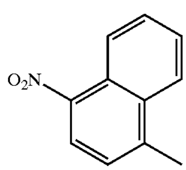
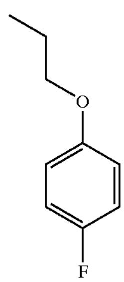
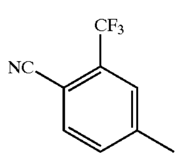
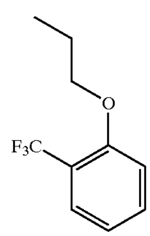
Ex. No	G	R⁷	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
280			(3aα,4β,7β,7aα)-4-[2-(4-Fluorophenoxy)ethyl]hexahydro-7-methyl-2-(3-methyl-4-nitrophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.68 LC	204, 35
281			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[4-[(trifluoromethyl)thio]phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	4.11 LC	204, 35
282			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-7-[2-(4-nitrophenoxy)ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.68 LC	204, 35
283			(3aα,4β,7β,7aα)-4-[2-(4-Fluorophenoxy)ethyl]hexahydro-7-methyl-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.68 & 3.80 rotational isomers LC	204, 35
284			(3aα,4β,7β,7aα)-4-[Octahydro-7-methyl-1,3-dioxo-7-[2-[2-(trifluoromethyl)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.89 LC	204, 35

TABLE 5-continued

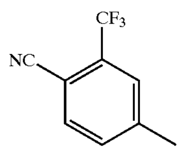
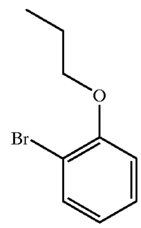
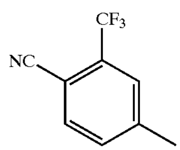
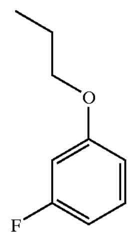
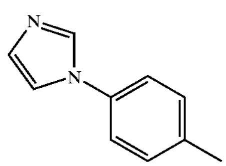
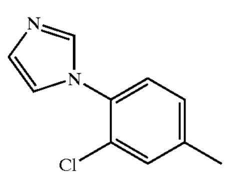
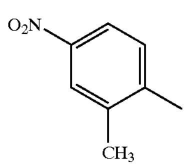
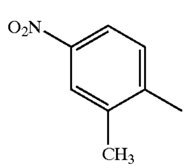
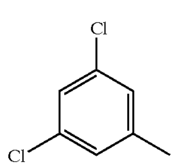
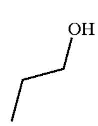
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
285			(3aα,4β,7β,7aα)-4-[4-[2-(2-Bromophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.91 LC	204, 35
286			(3aα,4β,7β,7aα)-4-[4-[2-(3-Fluorophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.78 LC	204, 35
287		H	(3aα,4β,7β,7aα)-Hexahydro-2-[4-(1H-imidazol-1-yl)phenyl]-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	1.16 LC	3
288		H	(3aα,4β,7β,7aα)-2-[3-Chloro-4-(2-thiazolyl) phenyl]hexahydro-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.81 LC	3
289		CH_3	(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-(3-methyl-4-nitrophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.74 LC	20
290		CH_3	(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-(2-methyl-4-nitrophenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.71 LC	20
291			(3aα,4β,7β,7aα)-2-(3,5-Dichlorophenyl)hexahydro-4-(2-hydroxyethyl)-7-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.98 LC	204

TABLE 5-continued

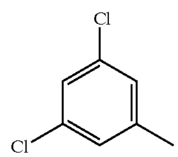
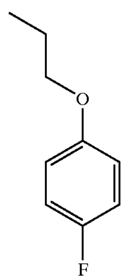
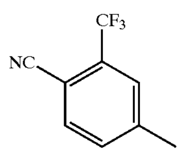
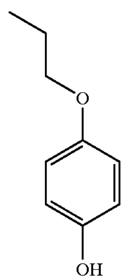
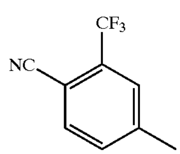
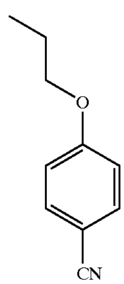
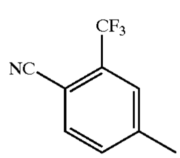
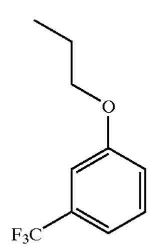
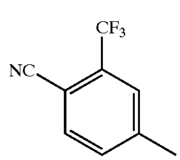
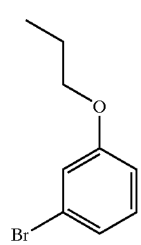
Ex. No	G	R⁷	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
292			(3aα,4β,7β,7aα)-2-(3,5-Dichlorophenyl)-4-[2-(4-fluorophenoxy)ethyl] hexahydro-7-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	4.03 LC	204, 35
293			(3aα,4β,7β,7aα)-4-[Octahydro-4-[2-(4-hydroxyphenoxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.25 LC	204, 35
294			(3aα,4β,7β,7aα)-4-[4-[2-(4-Cyanophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.51 LC	204, 35
295			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[3-(trifluoromethyl) phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.85 LC	204, 35
296			(3aα,4β,7β,7aα)-4-[4-[2-(3-Bromophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.84 LC	204, 35

TABLE 5-continued

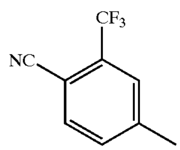
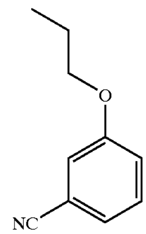
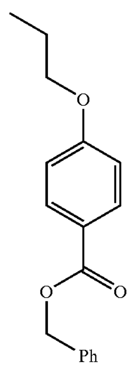
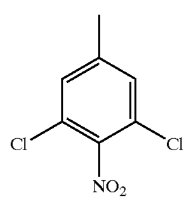
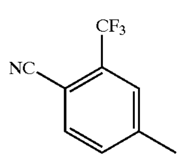
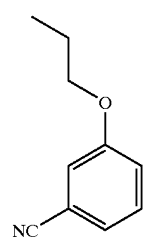
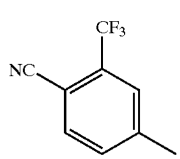
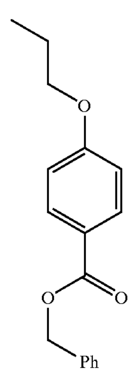
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
297			(3aα,4β,7β,7aα)-4-[4-[(4-Fluorophenyl)methyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.73 LC	205
298		CH_3	(3aα,4β,7β,7aα)-2-(1,6-Dihydro-1-methyl-6-oxo-3-pyridinyl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	1.61 LC	20
299		CH_3	(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-(1-methyl-6-oxo-3-piperidinyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	1.73 LC	20
300			(3aα,4β,7β,7aα)-4-[4-[2-(3-Cyanophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.46 LC	204, 35
301			(3aα,4β,7β,7aα)-4-[2-[4-Cyano-3-(trifluoromethyl) phenyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl] ethoxy]benzoic acid, phenylmethyl ester.	4.01 LC	204, 35

TABLE 5-continued

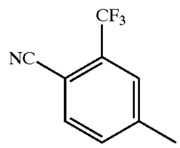
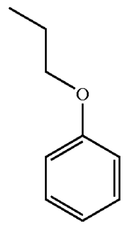
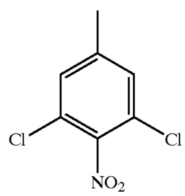
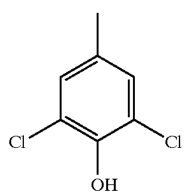
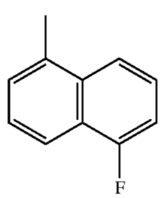
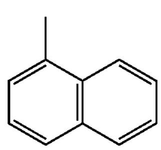
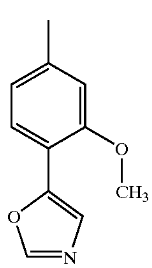
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
302			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-(2-phenoxyethyl)-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	3.57 LC	204, 35
303		CH_3	(3aα,4β,7β,7aα)-2-(3,5-Dichloro-4-nitrophenyl) hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.40 LC	20
304		CH_3	(3aα,4β,7β,7aα)-2-(3,5-Dichloro-4-hydroxyphenyl) hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.58 LC	20
305		CH_3	(3aα,4β,7β,7aα)-2-(5-Fluoro-1-naphthalenyl) hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.96 & 3.06 rotational isomers LC	20
306		CH_3	(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-(1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.60 & 2.73 rotational isomers LC	20
307		CH_3	(3aα,4β,7β,7aα)-Hexahydro-2-[3-methoxy-4-(5-oxazolyl)phenyl]-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.62 LC	20

TABLE 5-continued

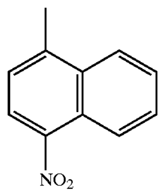
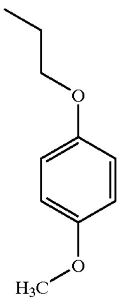
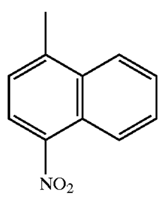
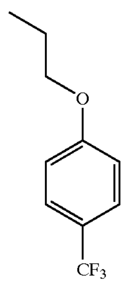
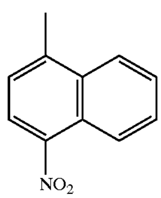
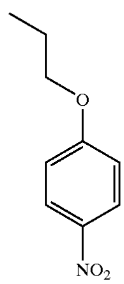
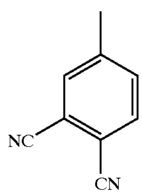
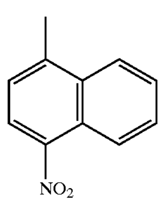
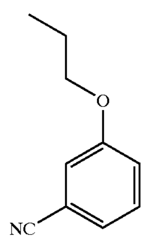
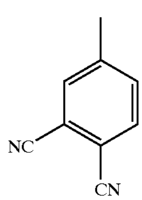
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
308			(3aα,4β,7β,7aα)-Hexahydro-4-[2-(4-methoxyphenoxy)ethyl]-7-methyl-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.42 & 3.55 rotational isomers LC	204, 35
309			(3aα,4β,7β,7aα)-Hexahydro-4-methyl-2-(4-nitro-1-naphthalenyl)-7-[2-[4-(trifluoromethyl)phenoxy]ethyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.81 & 3.93 rotational isomers LC	204, 35
310			(3aα,4β,7β,7aα)-Hexahydro-4-methyl-2-(4-nitro-1-naphthalenyl)-7-[2-(4-nitrophenoxy)ethyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.48 & 3.61 rotational isomers LC	204, 35
311		CH_3	(3aα,4β,7β,7aα)-2-(1,6-Dihydro-1,4-dimethyl-6-oxo-3-pyridinyl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	1.89 LC	20
312			(3aα,4β,7β,7aα)-4-[Octahydro-7-methyl-2-(4-nitro-1-naphthalenyl)-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy]benzonitrile.	3.63 LC	204, 35
313		CH_3	(3aα,4β,7β,7aα)-4-(Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-1,2-benzenedicarbonitrile.	2.38 LC	20

TABLE 5-continued

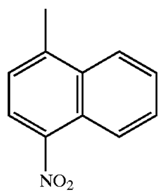
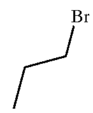
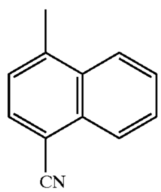
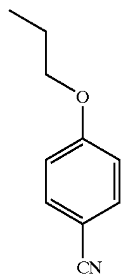
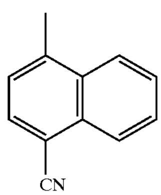
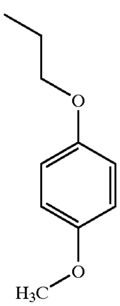
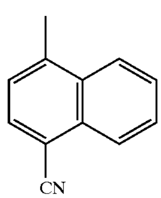
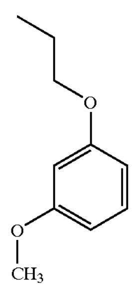
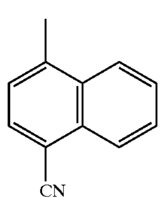
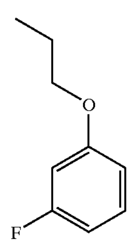
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
314			(3aα,4β,7β,7aα)-4-(2-Bromoethyl)hexahydro-7-methyl-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.52 LC	36
315			(3aα,4β,7β,7aα)-4-[4-[2-(4-Cyanophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.19 & 3.35 rotational isomers LC	223, 35
316			(3aα,4β,7β,7aα)-4-[Octahydro-4-[2-(4-methoxyphenoxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.34 & 3.50 rotational isomers LC	223, 35
317			(3aα,4β,7β,7aα)-4-[Octahydro-4-[2-(3-methoxyphenoxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.34 & 3.50 rotational isomers LC	223, 35
318			(3aα,4β,7β,7aα)-4-[4-[2-(3-Fluorophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.46 & 3.61 rotational isomers LC	223, 35

TABLE 5-continued

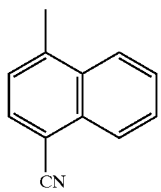
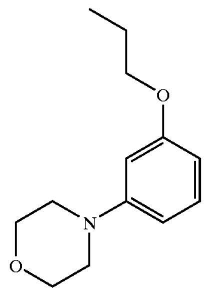
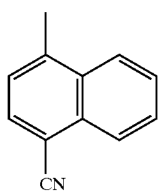
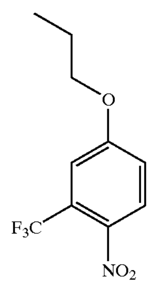
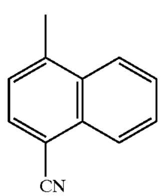
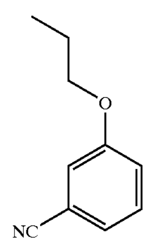
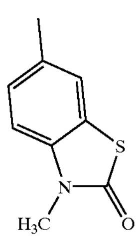
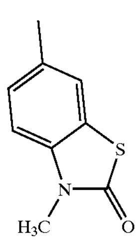
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
319			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-7-[2-[3-(4-morpholinyl)phenoxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.01 & 3.18 rotational isomers LC	223, 35
320			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-7-[2-[4-nitro-3-(trifluoromethyl)phenoxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.70 & 3.83 rotational isomers LC	223, 35
321			(3aα,4β,7β,7aα)-4-[4-[2-(3-Cyanophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.39 & 3.55 rotational isomers LC	223, 35
322		CH_3	(3aα,4β,7β,7aα)-2-(2,3-Dihydro-3-methyl-2-oxo-6-benzothiazolyl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.34 LC	20
323		CH_3	(3aα,4β,7β,7aα)-2-(2,3-Dihydro-2-oxo-6-benzothiazolyl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.16 LC	20

TABLE 5-continued

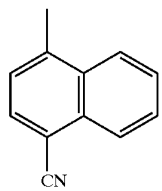
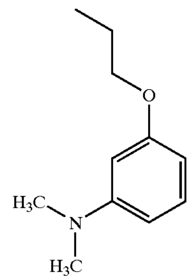
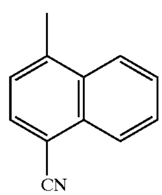
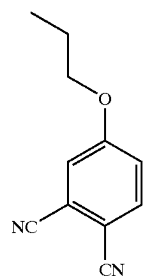
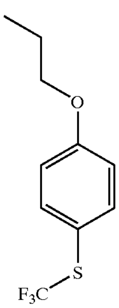
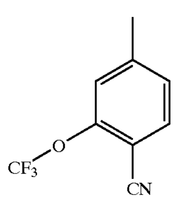
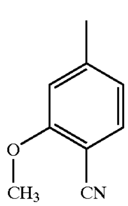
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
324			(3aα,4β,7β,7aα)-4-[4-[2-[3-(Dimethylamino)phenoxy] ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	2.63 & 2.79 rotational isomers LC	223, 35
325			(3aα,4β,7β,7aα)-4-[2-[4-Cyano-3-(trifluoromethyl) phenyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy]-1,2-benzenedicarbonitrile.	3.42 LC	223, 35
326		CH_3	(3aα,4β,7β,7aα)-N-[2-Cyano-5-(octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl) phenyl]acetamide.	1.94 LC	20
327		CH_3	(3aα,4β,7β,7aα)-4-(Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethoxy)benzonitrile.	3.52 LC	20
328		CH_3	(3aα,4β,7β,7aα)-2-Methoxy-4-(octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)benzonitrile.	2.47 LC	20

TABLE 5-continued

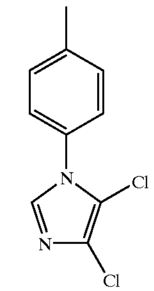
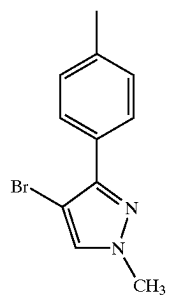
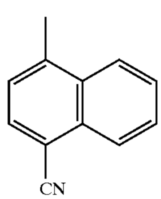
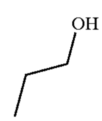
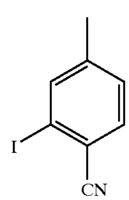
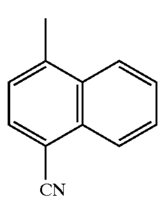
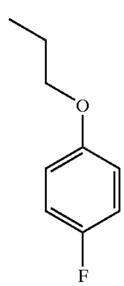
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
329		CH_3	(3aα,4β,7β,7aα)-2-[4-(4,5-Dichloro-1H-imidazol-1-yl)phenyl]hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.09 LC	20
330		CH_3	(3aα,4β,7β,7aα)-2-[4-(4-Bromo-1-methyl-1H-pyrazol-3-yl)phenyl]hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.04 LC	20
331			(3aα,4β,7β,7aα)-4-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	2.44 & 2.60 rotational isomers LC	223
332		CH_3	(3aα,4β,7β,7aα)-2-Iodo-4-(Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)benzonitrile.	2.78 LC	20
333			(3aα,4β,7β,7aα)-4-[4-[2-(4-Fluorophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.39 & 3.53 rotational isomers LC	223, 35

TABLE 5-continued

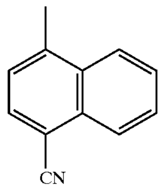
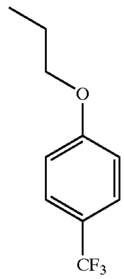
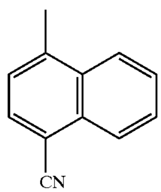
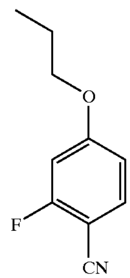
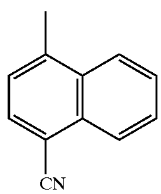
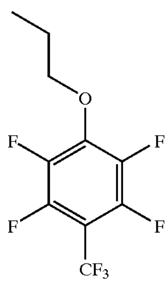
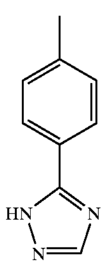
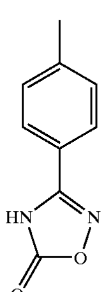
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
334			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[4-(trifluoromethyl)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.66 & 3.78 rotational isomers LC	223, 35
335			(3aα,4β,7β,7aα)-4-[4-[2-(4-Cyano-3-fluorophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-napthalenecarbonitrile.	3.26 & 3.41 rotational isomers LC	223, 35
336			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.94 & 4.01 rotational isomers LC	223, 35
337		CH_3	(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-[4-(1H-1,2,4-triazol-3-yl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.06 LC	20
338		CH_3	(3aα,4β,7β,7aα)-2-[4-(4,5-Dihydro-5-oxo-1,2,4-oxadiazol-3-yl)phenyl]hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.42 LC	20

TABLE 5-continued

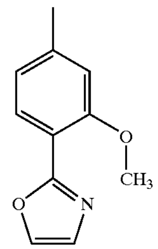
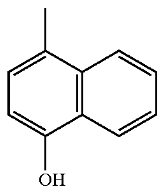
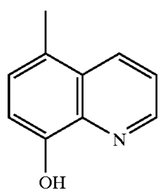
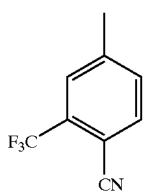
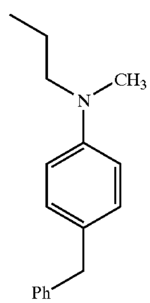
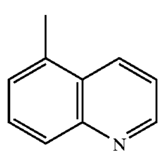
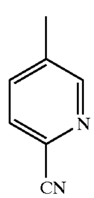
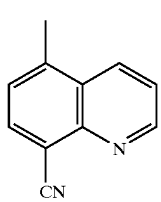
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
339		CH_3	(3aα,4β,7β,7aα)-Hexahydro-2-[3-methoxy-4-(2-oxazolyl)phenyl]-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.51 LC	20
340		CH_3	(3aα,4β,7β,7aα)-Hexahydro-2-(4-hydroxy-1-naphthalenyl)-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.30 LC	20
341		CH_3	(3aα,4β,7β,7aα)-Hexahydro-2-(8-hydroxy-5-quinolinyl)-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione, trifluoroacetate (1:1).	1.49 LC	20
342			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[methyl(phenylmethyl)amino]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile.	2.42 LC	204, 35
343		CH_3	(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-(5-quinolinyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	1.69 LC	20
344		CH_3	(3aα,4β,7β,7aα)-5-(Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-pyridinecarbonitrile.	2.18 LC	20
345		CH_3	(3aα,4β,7β,7aα)-5-(Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-8-quinolinecarbonitrile.	2.31 LC	20

TABLE 5-continued

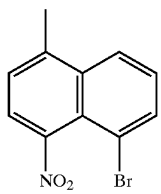
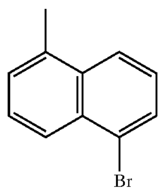
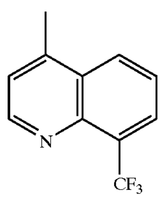
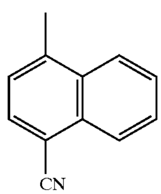
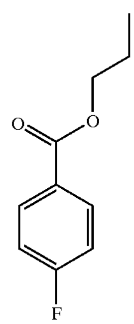
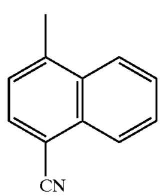
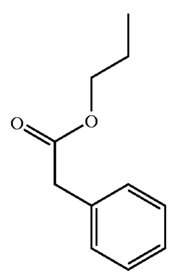
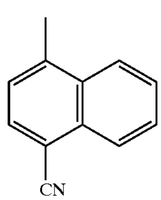
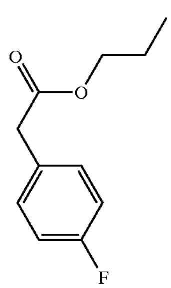
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
346		CH_3	(3aα,4β,7β,7aα)-2-(5-Bromo-4-nitro-1-naphthalenyl) hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.10 & 3.29 rotational isomers LC	20
347		CH_3	(3aα,4β,7β,7aα)-2-(5-Bromo-1-naphthalenyl) hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.28 & 3.40 rotational isomers LC	20
348		CH_3	(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-[8-(trifluoromethyl)-4-quinolinyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.08 LC	20
349			4-Fluorobenzoic acid, 2-[(3aα,4β,7β,7aα)-2-(4-cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-y]ethyl ester.	3.64 & 3.77 rotational isomers LC	223
350			Benzeneacetic acid, 2-[(3aα, 4β,7β,7aα)-2-(4-cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-y]ethyl ester.	3.53 & 3.67 rotational isomers LC	223
351			4-Fluorobenzeneacetic acid, 2-[(3aα,4β,7β,7aα)-2-(4-cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-y]ethyl ester.	3.53 & 3.66 rotational isomers LC	223

TABLE 5-continued

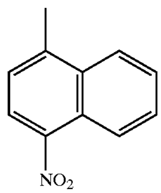
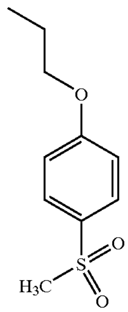
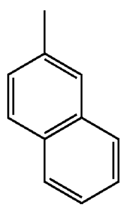
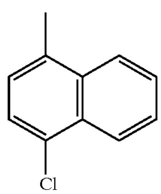
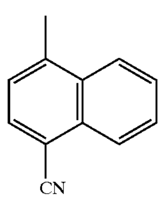
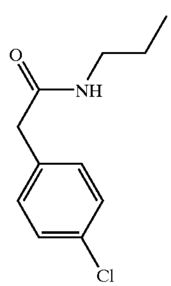
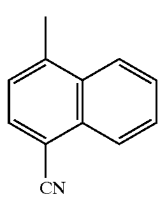
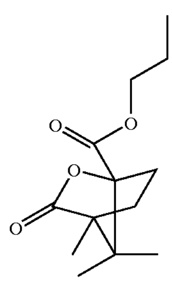
Ex. No	G	R7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
352			(3aα,4β,7β,7aα)-Hexahydro-4-methyl-7-[2-[4-(methylsulfonyl)phenoxy]ethyl]-2-(4-nitro-1-naphthalenyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.31 LC	204, 35
353		CH_3	(3aα,4β,7β,7aα)-Hexahydro-2-(2-naphthalenyl)-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.94 LC	20
354		CH_3	(3aα,4β,7β,7aα)-2-(4-Chloro-1-naphthalenyl) hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.22 & 3.34 rotational isomers LC	20
355			(3aα,4β,7β,7aα)-N-[(4-Chlorophenyl)methyl]-2-(4-cyano-1-naphthalenyl) octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindole-4-acetamide.	3.52 LC	237
356			4,7,7-Trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxylic acid, 2-[(3aα,4β,7β,7aα)-2-(4-cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-y]ethyl ester.	3.45 LC	223

TABLE 5-continued

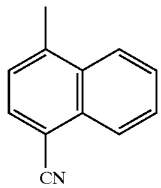
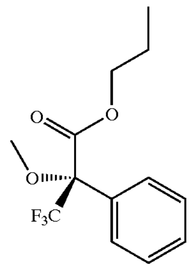
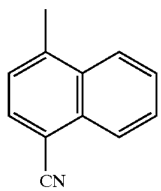
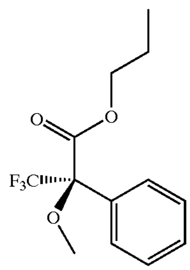
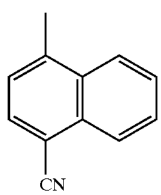
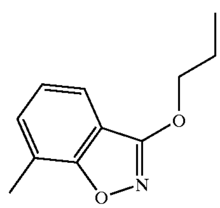
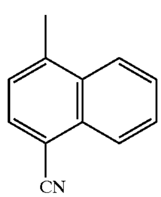
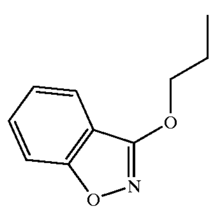
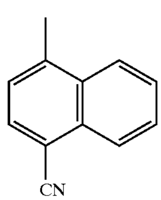
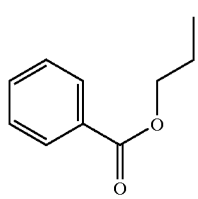
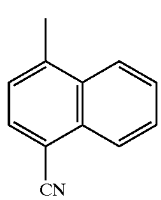
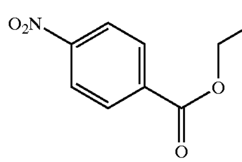
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
357			(αS)-α-Methoxy-α-(trifluoromethyl)benzeneacetic acid, 2-[(3aα,4β,7β,7aα)-2-(4-cyano-1-naphthalenyl) octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-y]ethyl ester.	3.91 LC	223
358			(αR)-α-Methoxy-α-(trifluoromethyl)benzeneacetic acid, 2-[(3aα,4β,7β,7aα)-2-(4-cyano-1-naphthalenyl) octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-y]ethyl ester.	2.00 LC	223
359			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-7-[2-[(7-methyl-1,2-benzisoxazol-3-yl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.79 & 3.92 LC Rotationale isomers	250
360			(3aα,4β,7β,7aα)-4-[4-[2-(1,2-Benzisoxazol-3-yloxy) ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.55 & 3.70 LC Rotationale Isomers	250
361			(3aα,4β,7β,7aα)-4-[2-(Benzoyloxy)ethyl]-2-(4-cyano-1-naphthalenyl)hexahydro-7-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.51 & 3.66 LC Rotationale isomers	223
362			(3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl)-4-[2-[(4-nitrobenzoyl)oxy]ethyl] hexahydro-7-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	3.52 & 3.67 LC Rotationale Isomers	223

TABLE 5-continued

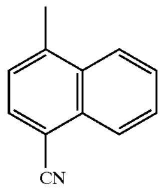
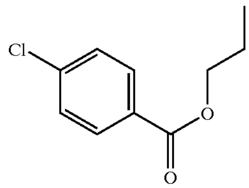
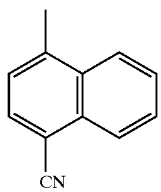
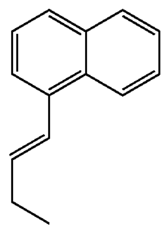
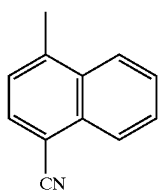
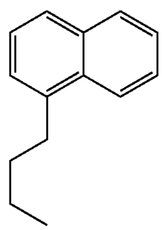
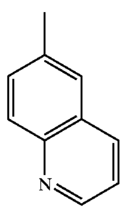
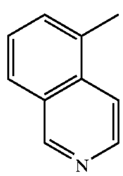
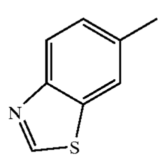
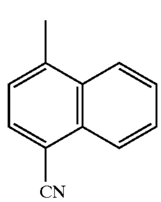
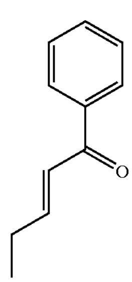
Ex. No	G	R⁷	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
363			4-Chlorobenzoic acid, 2-[(3aα,4β,7β,7aα)-2-(4-cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-y]ethyl ester.	3.79 LC	223
364			[3aα,4β,7β,7aα(E)]-4-[Octahydro-4-methyl-7-[3-(1-naphthalenyl)-2-propenyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	4.14 LC 499.13 $[M + H]^+$	248
365			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-7-[3-(1-naphthalenyl)propyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	4.14 LC 501.14 $[M + H]^+$	248, 249
366		CH_3	(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-(2-methyl-6-quinolinyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	1.25 LC 337.0 $[M + H]^+$	20
367		CH_3	(3aα,4β,7β,7aα)-Hexahydro-2-(5-isoquinolinyl)-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	1.06 & 1.29 LC Rotationale Isomers 323.0 $[M + H]^+$	20
368		CH_3	(3aα,4β,7β,7aα)-2-(6-Benzothiazolyl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	2.15 LC 329.0 $[M + H]^+$	20
369			[3aα,4β,7β,7aα(E)]-4-[Octahydro-4-methyl-1,3-dioxo-7-(4-oxo-4-phenyl-2-butenyl)-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.50 LC 477.1 $[M + H]^+$	265

TABLE 5-continued

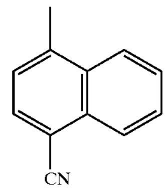
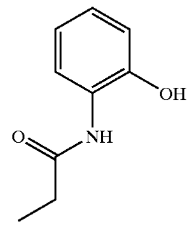
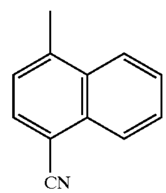
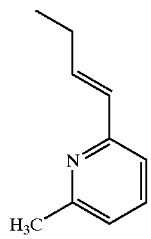
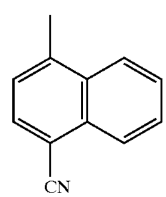
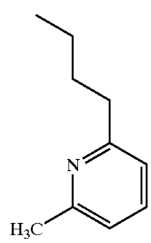
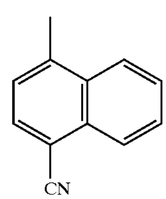
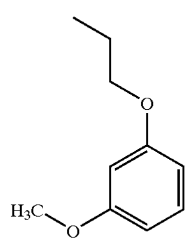
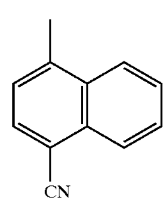
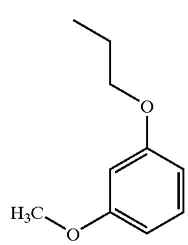
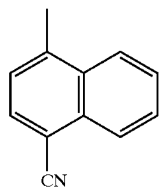
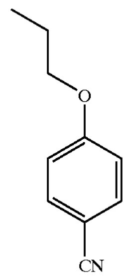
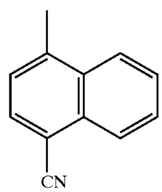
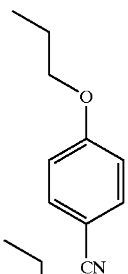
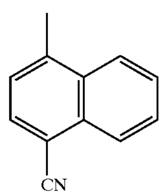
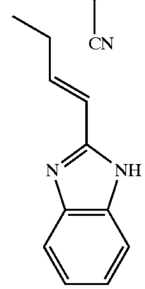
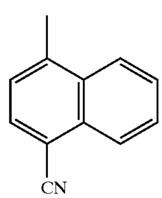
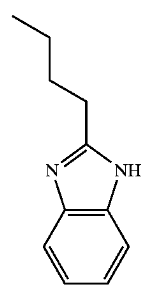
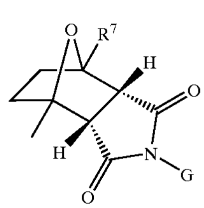
Ex. No	G	R⁷	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
370			(3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl)octahydro-N-(2-hydroxyphenyl)-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindole-4-acetamide.	3.07 LC 482.14 $[M + H]^+$	236
371			[3aα,4β(E),7β,7aα]-4-[Octahydro-4-methyl-7-[3-(6-methyl-2-pyridinyl)-2-propenyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	2.28 LC 464.19 $[M + H]^+$	248
372			(3aα,4β,7β,7aα)-4-[Octahydro-4-methyl-7-[3-(6-methyl-2-pyridinyl)propyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	2.19 LC 466.32 $[M + H]^+$	248, 249
373			[3aR-(3aα,4β,7β,7aα]-4-[Octahydro-4-[2-(3-methoxyphenoxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.73 LC 483.65 $[M + H]^+$	238i, 239i
374			[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-(3-methoxy phenoxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.73 LC	238ii, 239ii

TABLE 5-continued

Ex. No	G	R⁷	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
375			[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(4-Cyanophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.33 & 3.49 LC Rotationale Isomers	238i, 239i
376			[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(4-Cyanophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	3.73 LC 483.65 [M + H]⁺	238ii, 239ii
377			[3aα,4β(E),7β,7aα]-4-[4-[3-(1H-Benzimidazol-2-yl)-2-propenyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile.	2.48 LC 489.26 [M + H]⁺	248
378			(3aα,4β,7β,7aα)-4-[4-[3-(1H-Benzimidazol-2-yl)propyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.37 LC 491.26 [M + H]⁺	249

EXAMPLES 379 TO 381

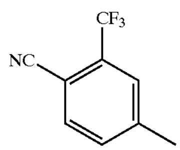
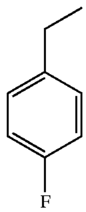
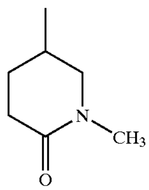
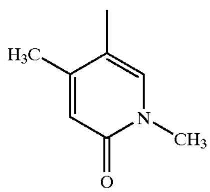
Additional compounds of the present invention were prepared by procedures analogous to those described above. The compounds of Examples 379 to 381 have the following structure (L is a bond):



where G, R^7, the compound name, retention time, molecular mass, and the procedure employed, are set forth in Table 6. The chromatography techniques used to determine the compound retention times of Table 6 are as follows: LCMS=YMC S5 ODS column, 4.6×50 mm eluting with 10–90% MeOH/H_2O over 4 minutes containing 0.1% TFA; 4 mL/min, monitoring at 220 nm. LCMS*=YMC S5 ODS column, 4.6×50 mm eluting with 10–90% MeOH/H_2O over 2 minutes containing 0.1% TFA; 4 mL/min, monitoring at 220 nm. LC=YMC S5 ODS column 4.6×50 mm eluting with 10–90% MeOH/H_2O over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm.

The molecular mass of the compounds listed in Table 6 were determined by MS (ES) by the formula m/z.

TABLE 6

Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
379			(3aα,4α,7α,7aα)-4-[4-[(4-Fluorophenyl)-methyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl) benzonitrile.	3.75 LC	205
380		CH_3	(3aα,4α,7α,7aα-Hexahydro-4,7-dimethyl-2-(1-methyl-6-oxo-3-piperidinyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	1.88 LC	27
381		CH_3	(3aα,4α,7α,7aα), 2-(1,6-Dihydro-1,4-dimethyl-6-oxo-3-pyridinyl)hexa-hydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione.	1.91 LC	27

EXAMPLES 382 TO 383

Additional compounds of the present invention were prepared by procedures analogous to those described above. The compounds of Examples 382 to 383 have the structure, compound name, retention time, molecular mass, and were prepared by the procedure employed, set forth in the following Table 7. The chromatography techniques used to determine the compound retention times of Table 7 are as follows: LCMS=YMC S5 ODS column, 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 4 minutes containing 0.1% TFA; 4 mL/min, monitoring at 220 nm. LCMS*=YMC S5 ODS column, 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 2 minutes containing 0.1% TFA; 4 mL/min, monitoring at 220 nm. LC=YMC S5 ODS column 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm. The molecular mass of the compounds listed in Table 7 were determined by MS (ES) by the formula m/z.

TABLE 7

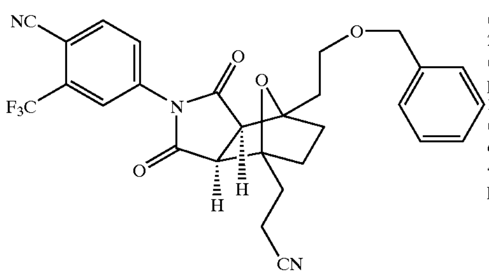
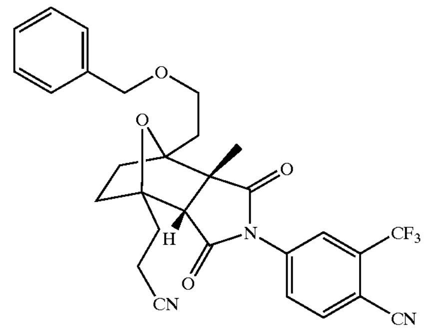
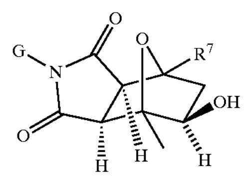
Ex. No.	Structure	Compound Name	Retention Time Min.	Procedure of Example
382		(3aα,4β,7β,7aα)-2-[4-Cyano-3-(trifluoromethyl) phenyl]octahydro-1,3-dioxo-7-[2-(phenylmethoxy) ethyl]-4,7-epoxy-4H-isoindole-4-propanenitrile.	3.63 LC	255

TABLE 7-continued

Ex. No.	Structure	Compound Name	Retention Time Min.	Procedure of Example
383		(3aα,4β,7β,7aα)-2-[4-Cyano-3-(trifluoromethyl)phenyl]octahydro-1,3-dioxo-7-[2-(phenylmethoxy)ethyl]-4,7-epoxy-4H-isoindole-4-propanenitrile	3.64 LC	255

EXAMPLES 384 TO 418

Additional compounds of the present invention were prepared by procedures analogous to those described above. The compounds of Examples 384 to 418 have the following structure (L is a bond):



where G, R^7, the compound name, retention time, molecular mass, and the procedure employed, are set forth in Table 8. The absolute configuration for the following compounds was not determined. For simplicity in nomenclature, compound 243Di is designated herein as having an "S" configuration and compound 243Dii as having an "R" configuration. Enantiomerically pure products derived from compound 243Di are designated herein as having an "S" configuration and enantiomerically pure products derived from compound 243Dii are designated herein as having an "R" configuration.

The chromatography techniques used to determine the compound retention times of Table 8 are as follows: LCMS=YMC S5 ODS column, 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 4 minutes containing 0.1% TFA; 4 mL/min, monitoring at 220 nm. LCMS*=YMC S5 ODS column, 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 2 minutes containing 0.1% TFA; 4 mL/min, monitoring at 220 nm. LC=YMC S5 ODS column 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm. The molecular mass of the compounds listed in Table 8 were determined by MS (ES) by the formula m/z.

TABLE 8

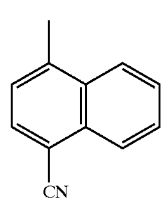
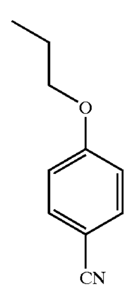
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
384			(3aα,4β,7β,7aα)-4-[7-[2-(4-Cyanophenoxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphtalene-carbonitrile.	3.18 LC 494.40 $[M + H]^+$	227, 228, 229

TABLE 8-continued

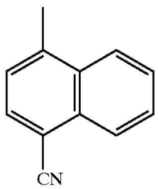
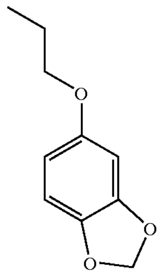
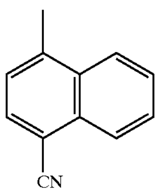
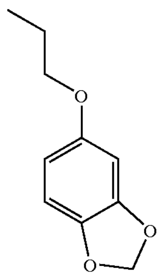
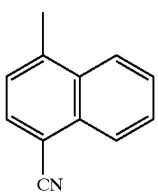
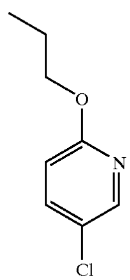
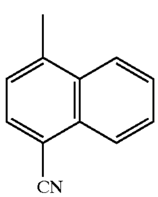
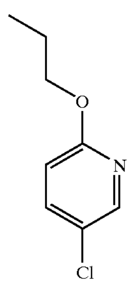
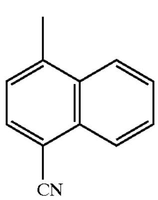
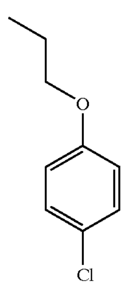
Ex. No	G	R⁷	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
385			[3aS-(3aα,4β,7β,7aα)]-4-[7-[2-(1,3-Benzodioxol-5-yloxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.19 LC 571.3 $[M - H + OAc]^-$	243Di, 244i
386			[3aR-(3aα,4β,7β,7aα)]-4-[7-[2-(1,3-Benzodioxol-5-yloxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.22 LC 571.2 $[M - H + OAc]^-$	234Dii, 244ii
387			[3aS-(3aα,4β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.37 LC 562.2 $[M - H + OAc]^-$	243Di, 244i
388			[3aR-(3aα,4β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.37 LC 504.0 $[M + H]^+$	243Dii, 244ii
389			[3aS-(3aα,4β,7β,7aα)]-4-[7-[2-(4-Chlorophenoxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.51 LC 503.08 $[M + H]^+$	243Di, 244i

TABLE 8-continued

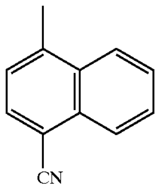
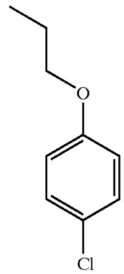
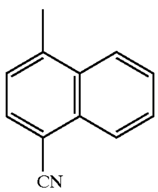
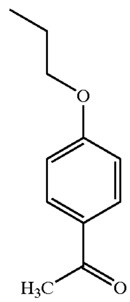
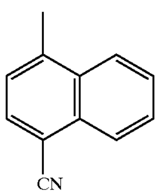
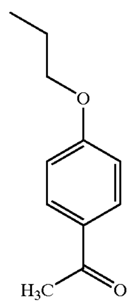
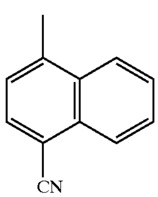
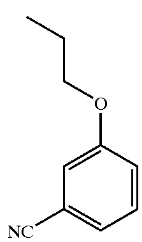
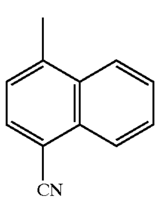
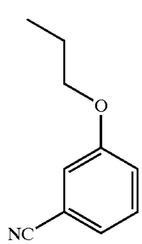
Ex. No	G	R7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
390			[3aR-(3aα,4β,7β,7aα)]-4-[7-[2-(4-Chlorophenoxy) ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.51 LC 503.08 $[M + H]^+$	243Dii, 244ii
391			[3aS-(3aα,4β,7β,7aα)]-4-[7-[2-(4-Acetylphenoxy) ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.05 LC 511.13 $[M + H]^+$	243Di, 244i
392			[3aR-(3aα,4β,7β,7aα)]-4-[7-[2-(4-Acetylphenoxy) ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.05 LC 503.13 $[M + H]^+$	243Dii, 244ii
393			[3aS-(3aα,4β,7β,7aα)]-4-[7-[2-(3-Cyanophenoxy) ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.09 LC 494.13 $[M + H]^+$	243Di, 244i
394			[3aR-(3aα,4β,7β,7aα)]-4-[7-[2-(3-Cyanophenoxy) ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.09 LC 494.13 $[M + H]^+$	243Dii, 244ii

TABLE 8-continued

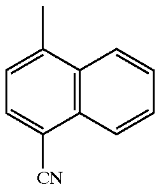
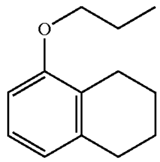
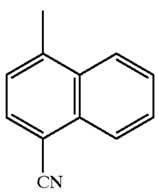
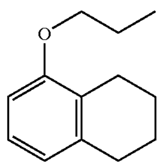
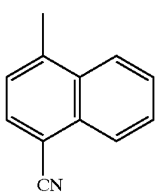
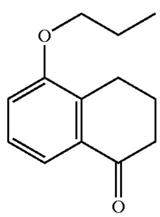
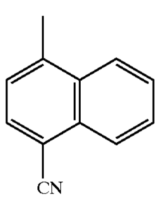
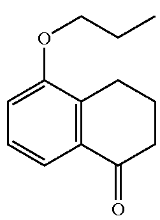
Ex. No	G	R⁷	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
395			[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[(5,6,7,8-tetrahydro-1-naphthalenyl)oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.85 LC 523.17 $[M + H]^+$	243Di, 244i
396			[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[(5,6,7,8-tetrahydro-1-naphthalenyl)oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.85 LC 523.17 $[M + H]^+$	243Dii, 244ii
397			[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[(5,6,7,8-tetrahydro-5-oxo-1-naphthalenyl)oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.29 LC 537.13 $[M + H]^+$	243Di, 244i
398			[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[(5,6,7,8-tetrahydro-5-oxo-1-naphthalenyl)oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.29 LC 537.13 $[M + H]^+$	243Dii, 244ii

TABLE 8-continued

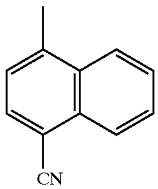
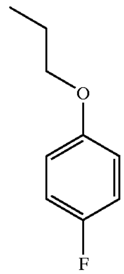
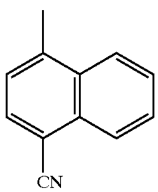
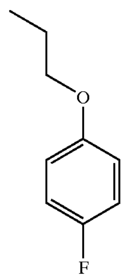
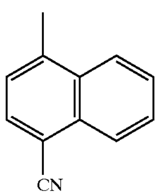
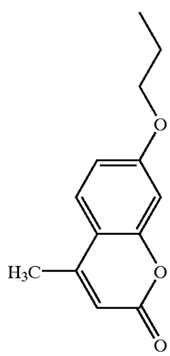
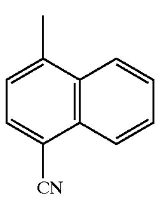
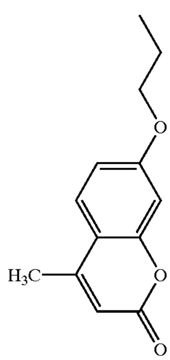
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
399			[3aS-(3aα,4β,7β,7aα)]-4-[7-[2-(4-Fluorophenoxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.28 LC 487.11 $[M + H]^+$	243Di, 244i
400			[3aR-(3aα,4β,7β,7aα)]-4-[7-[2-(4-Fluorophenoxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.27 LC 487.11 $[M + H]^+$	243Dii, 244ii
401			[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-7-[2-[(4-methyl-2-oxo-2H-1-benzopyran-7-yl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.15 LC 551.15 $[M + H]^+$	243Di, 244i
402			[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-7-[2-[(4-methyl-2-oxo-2H-1-benzopyran-7-yl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.16 LC 551.10 $[M + H]^+$	243Dii, 244ii

TABLE 8-continued

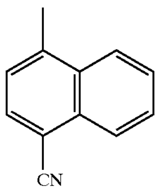
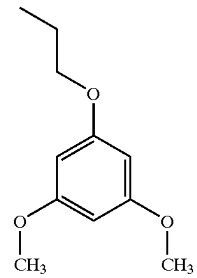
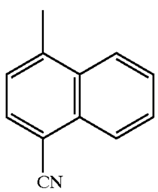
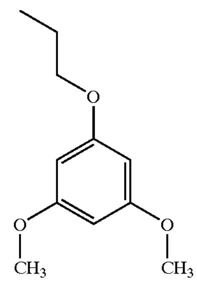
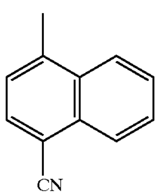
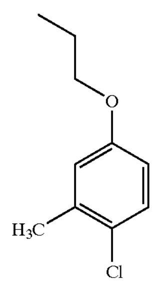
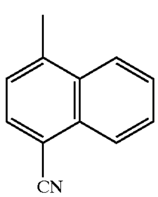
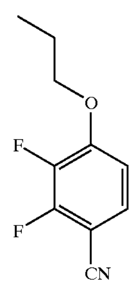
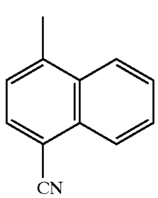
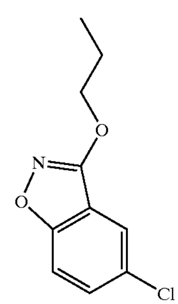
Ex. No	G	R⁷	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
403			[3aS-(3aα,4β,7β,7aα)]-4-[7-[2-(3,5-Dimethoxyphenoxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2--yl]-1-naphthalene-carbonitrile.	3.28 LC 529.19 $[M + H]^+$	243Di, 244i
404			[3aR-(3aα,4β,7β,7aα)]-4-[7-[2-(3,5-Dimethoxyphenoxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2--yl]-1-naphthalene-carbonitrile.	3.26 LC 529.12 $[M + H]^+$	243Dii, 244ii
405			[3aR-(3aα,4β,7β,7aα)]-]-4-[7-[2-(4-Chloro-3-methylphenoxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2--yl]-1-naphthalene-carbonitrile.	3.68 LC 517.33 $[M + H]^+$	243Dii, 244ii
406			[3aR-(3aα,4β,7β,7aα)]-4-[7-[2-(4-Cyano-2,3-difluorophenoxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.23 LC 530.13 $[M + H]^+$	243Dii, 244ii
407			[3aS-(3aα,4β,7β,7aα)]-4-[7-[2-[(5-Chloro-1,2-benzisoxazol-3-yl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.59 LC 602.1 $[M - H + OAc]^-$	243Di, 252

TABLE 8-continued

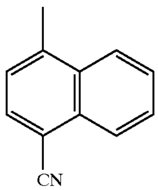
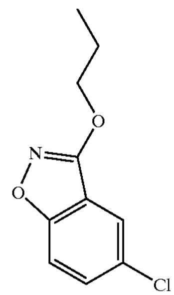
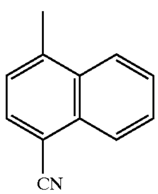
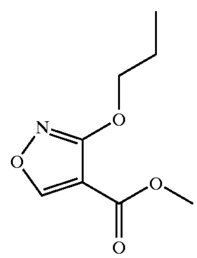
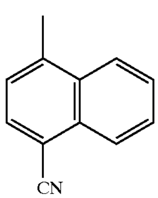
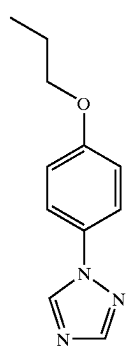
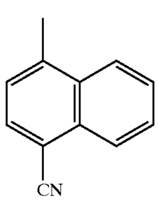
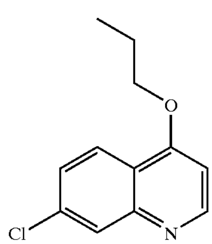
Ex. No	G	R^7	Compound Name	Retention Time Min./Molecular Mass	Procedure of Ex.
408			[3aR-(3aα,4β,7β,7aα)]-4-[7-[2-[(5-Chloro-1,2-benzisoxazol-3-yl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.57 LC 602.0 $[M - H + OAc]^-$	243Dii, 253
409			[3aR-(3aα,4β,7β,7aα)]-3-[2-[2-(4-Cyano-1-naphthalenyl)octahydro-6-hydroxy-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy]-5-isoxazolecarboxylic acid, methyl ester.	2.90 LC 518.27 $[M + H]^+$	243Dii, 253
410			[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[4-(1H-1,2,4-triazol-1-yl)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	2.93 LC 536.30 $[M + H]^+$	243Dii, 244ii
411			[3aS-(3aα,4β,7β,7aα)]-4-[7-[2-[(7-Chloro-4-quinolinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile, trifluoroacetate (1:1).	2.52 LC 554.13 $[M + H]^+$	243Di, 244i

TABLE 8-continued

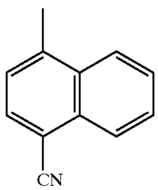
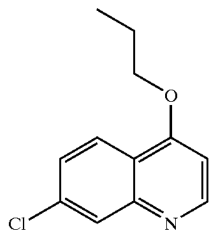
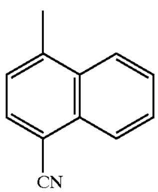
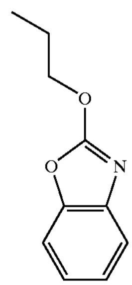
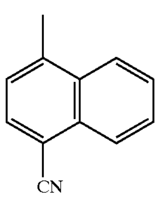
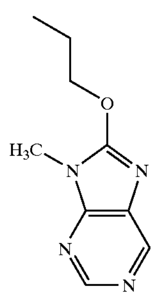
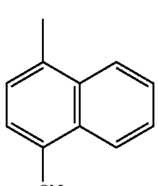
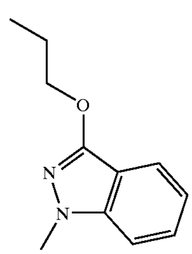
Ex. No	G	R7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
412			[3aR-(3aα,4β,7β,7aα)]-4-[7-[2-[(7-Chloro-4-quinolinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile, trifluoroacetate (1:1).	2.53 LC 554.27 $[M + H]^+$	243Dii, 244ii
413			[3aR-(3α,4β,5β,7β,7aα)]-4-[7-[2-(2-Benzoxazolyloxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.13 LC 568.1 $[M - H + OAc]^-$	243Dii, 244ii
414			[3aR-(3α,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-7-[2-[(9-methyl-9H-purin-8-yl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	2.34 LC 525.2 $[M + H]^+$	243Dii, 244ii
415			[3aR-(3α,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-7-[2-[(1-methyl-1H-indazol-3-yl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.33 LC	251, 253

TABLE 8-continued

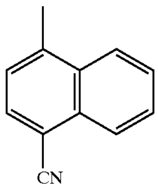
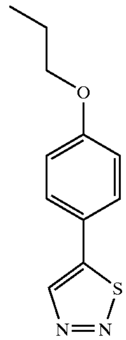
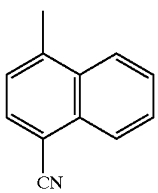
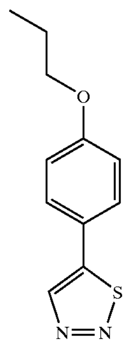
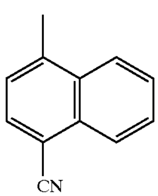
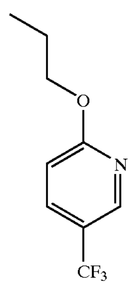
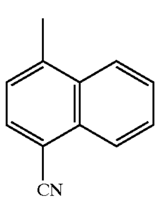
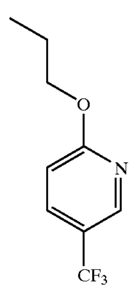
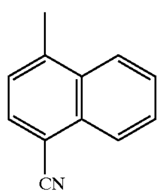
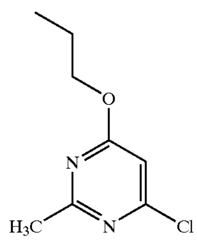
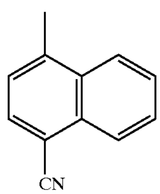
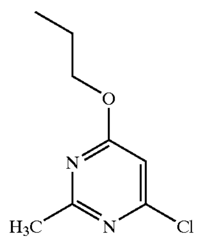
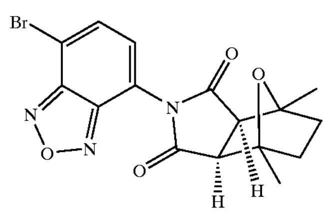
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
416			[3aS-(3α,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-7-[2-[4-(1,2,3-thiadiazol-5-yl)phenoxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.17 LC 553.10 $[M + H]^+$	243Dii, 244ii
417			[3aR-(3α,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-7-[2-[4-(1,2,3-thiadiazol-5-yl)phenoxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.20 LC 553.25 $[M + H]^+$	243Dii, 244ii
418			[3aS-(3α,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[[5-(trifluoromethyl)-2-pyridinyl]oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.45 LC 538.23 $[M + H]^+$	243Dii, 244ii
419			[3aR-(3α,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[[5-(trifluoromethyl)-2-pyridinyl]oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.45 LC 538.23 $[M + H]^+$	243Dii, 244ii

TABLE 8-continued

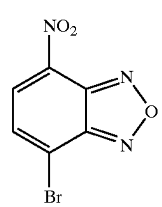
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
420			[3aS-(3α,4β,5β,7β,7aα)]-4-[7-[2-[(6-Chloro-2-methyl-4-pyrimidinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.02 LC	243Dii, 244ii
421			[3aR-(3α,4β,5β,7β,7aα)]-4-[7-[2-[(6-Chloro-2-methyl-4-pyrimidinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile.	3.02 LC	243Dii, 244ii

EXAMPLE 422

(3aα,4β,7β,7aα)-2-(7-Bromo-2,1,3-benzoxadiazol-4-yl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (422C)

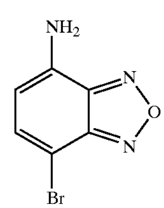


A. 4-Bromo-7-nitrobenzofurazan (422A)



To a solution of 2,6-dibromoaniline (1.0 g, 4.0 mmol) in $CHCl_3$ (8 mL) was added a suspension of mCPBA (70% by HPLC, 1.4 g, 8.0 mmol) in $CHCl_3$ (8 mL) and the resulting mixture was stirred for 24 h at rt. The reaction mixture was diluted with $CHCl_3$ and washed successively with 2% $Na_2S_2O_3$ solution, 5% Na_2CO_3 solution and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to leave a solid, which was suspended, into DMSO (15 mL). To this suspension was added a solution of NaN_3 (272 mg, 4.19 mmol) in DMSO (15 mL) at rt. The resulting mixture was stirred at rt until most of the nitrogen had evolved and was then quickly heated to 120° C. for 3 min. The reaction mixture was cooled and poured onto crushed ice (100 g). After standing for 1 h the precipitates were filtered off, dried in vacuo and redissolved in conc. H_2SO_4 (5 mL). To this solution was added a solution of $NaNO_3$ (400 mg, 4.7 mmol) in 50% H_2SO_4 (1.6 mL) and the temperature was maintained at 60° C. After the addition was complete, the mixture was heated at 85° C. for 30 min, cooled to rt and poured onto crushed ice (40 g). EtOAc was added, the layers were separated and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to leave a solid which was purified by flash chromatography (silica gel, EtOAc (20%) in hexanes) affording compound 422A (785 mg, 81%) as a tan solid.

B. 4-Bromo-7-aminobenzofurazan (422B)



A solution of compound 422A (563 mg, 2.31 mmol) in AcOH (5 mL) was heated to 70° C. and Fe^0 powder (258 mg, 4.62 mmol) was added in one portion. The resulting

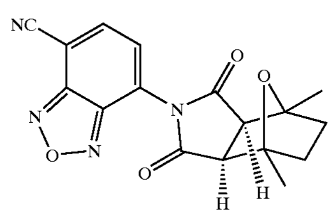
dark reaction mixture was stirred for 15 min, cooled to rt and concentrated under reduced pressure. The residue was taken up in EtOAc and the resulting solution was washed with sat. Na_2CO_3 solution. The organic layer was dried over Na_2SO_4, concentrated in vacuo and purified by flash chromatography on silica gel eluting with 10–60% EtOAc in hexanes to give 470 mg (95%) of compound 422B as a red solid.

C. (3aα,4β,7β,7aα)-2-(7-Bromo-2,1,3-benzoxadiazol-4-yl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (422C)

A mixture of compound 422B (43 mg, 0.20 mmol), compound 20A (45 mg, 0.23 mmol), $MgSO_4$ (60 mg, 0.50 mmol), Et_3N (139 μL, 1.0 mmol) and 1,2-dimethoxyethane (300 μL) were placed in a sealed tube and heated at 135° C. for 14 h. After cooling to rt the mixture was filtered through Celite eluting with MeOH to yield a dark solid which was purified by flash chromatography on silica gel eluting with 5–40% EtOAc in hexanes to give 42 mg (54%) of compound 422C as a yellow solid. HPLC: 99% at 2.96 min (retention time) (YMC S5 ODS column 4.6×50 mm Ballistic, 10–90% aqueous methanol over 4 minutes containing 0.2% H_3PO_4, 4 mL/min, monitoring at 220 nm). ^1H NMR (acetone-d_6, 400 MHz): δ=8.00 (d, J=7.5 Hz, 1H), 7.45 (d, J=7.5 Hz, 1H), 3.31 (s, 2H), 1.98–1.93 (m, 2H), 1.74–1.69 (m, 2H), 1.57 (s, 6H).

EXAMPLE 423

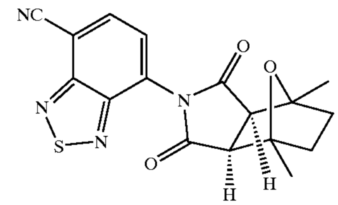
(3aα,4β,7β,7aα)-7-[Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzoxadiazole-4-carbonitrile (423)



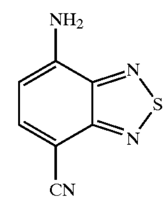
To a solution of compound 422C (42 mg, 0.11 mmol) in DMA (1 mL) was added CuCN (20 mg, 0.22 mmol) and the resulting mixture was heated at 150° C. for 5 h. The mixture was allowed to cool to rt and partitioned between EtOAc and aqueous NaCN solution (5 g/50 mL). The layers were separated and the aqueous layer was extracted once with EtOAc. The combined organic phases were dried over Na_2SO_4, concentrated in vacuo and purified by flash chromatography on silica gel eluting with 20–70% EtOAc in hexanes to give 13 mg (35%) of compound 423 as a yellow oil. HPLC: 99% at 2.66 min (retention time) (YMC S5 ODS column 4.6×50 mm Ballistic, 10–90% aqueous methanol over 4 minutes containing 0.2% H_3PO_4, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 396.9 $[M-H+OAc]^-$.

EXAMPLE 424

(3aα,4β,7β,7aα)-7-[Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile (424B)



A. 4-Cyano-7-amino-benzothiadiazole (424A)



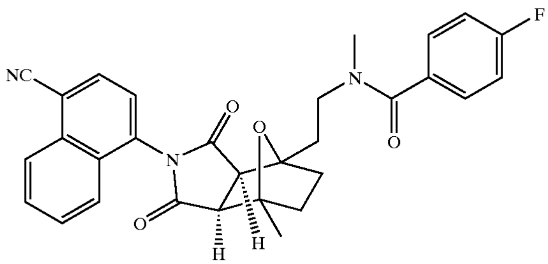
A solution of 2-cyano-5-nitrophenylenediamine (78 mg, 0.44 mmol, prepared as described in WO 0076501) in $SOCl_2$ (2 mL) was heated to reflux for 3 h. The resulting mixture was allowed to cool to rt and was then poured into ice/water. CH_2Cl_2 was added, the layers were separated and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, concentrated in vacuo and purified by flash chromatography on silica gel eluting with 50% EtOAc in hexanes to give 4-cyano-7-nitrobenzothiadiazole. This material was dissolved in AcOH (2 mL) containing EtOAc (1 mL) and H_2O (0.2 mL) and heated to 70° C. At this temperature Fe^0 powder (78 mg, 1.41 mmol) was added in one solid portion and the dark mixture was stirred for 20 min and then cooled to rt. The reaction mixture was filtered through Celite eluting with EtOAc, washed with sat. Na_2CO_3 solution, dried over $MgSO_4$ and concentrated in vacuo. Purification by flash chromatography on silica gel eluting with 20–70% EtOAc in hexanes to yield 47 mg (67%) of compound 424A as a brown solid.

B. (3aα,4β,7β,7aα)-7-[Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile (424B)

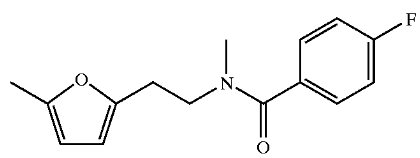
A mixture of compound 424A (35 mg, 0.20 mmol), compound 20A (45 mg, 0.23 mmol), $MgSO_4$ (60 mg, 0.50 mmol), Et_3N (139 μL, 1.0 mmol) and DME (200 μL) was placed in a sealed tube and heated at 135° C. for 14 h. After cooling to rt the mixture was filtered through Celite eluting with MeOH to yield a dark solid which was purified by a combination of flash chromatography on silica gel eluting with 10–50% EtOAc in hexanes reverse phase preparative HPLC (YMC S5 ODS 20×100 mm eluting with 27–100% aqueous methanol over 10 min containing 0.1% TFA, 20 mL/min) to give 36 mg (51%) of compound 424B as a yellow solid. HPLC: 98% at 2.45 min (retention time) (YMC S5 ODS column 4.6×50 mm Ballistic, 10–90% aqueous methanol over 4 minutes containing 0.2% H_3PO_4, 4 mL/min, monitoring at 220 nm). MS (DCI): m/z 355.0 $[M+H]^+$.

EXAMPLE 425

(3aα,4β,7β,7aα)-N-2-[2-(4-Cyano-1-naphthalenyl) octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethyl]-4-fluoro-N-methylbenzamide (425B)



A. 4-Fluoro-N-methyl-N-[2-(5-methyl-furan-2-yl)-ethyl]-benzamide (425A)



NaH (60% dispersion in oil, 65 mg, 1.63 mmol) was added portionwise to a solution of 4-fluoro-N-[2-(5-methyl-2-furanyl)ethyl]benzamide (269 mg, 1.09 mmol, 237A) in THF (5 mL). After gas evolution ceased, iodomethane (0.14 mL, 2.18 mmol) was added drop-wise. Once HPLC analysis showed the reaction to be 50% complete, the mixture was concentrated under reduced pressure and resubjected to the above conditions. After all the starting material was consumed, H_2O was added and the resulting mixture was extracted with EtOAc (2×5 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 20% acetone/$CHCl_3$ gave 238 mg (84%) of compound 425A. HPLC: 98% at 2.94 min (retention time) (Phenomenex-prime S5-C18 column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z [M+H]=262.38.

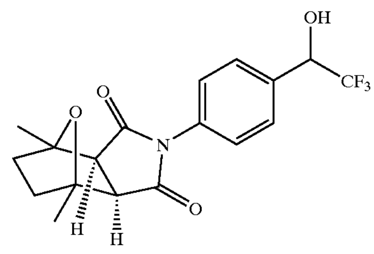
B. (3aα,4β,7β,7aα)-N-[2-[2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethyl]-4-fluoro-N-methylbenzamide (425B)

A solution of compound 425A (183 mg, 0.75 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-1-yl)-1-naphthalenecarbonitrile (174 mg, 0.75 mmol) in benzene (1 mL) was heated at 60° C. for 15 hr. The reaction mixture was concentrated under reduced pressure to give 357 mg crude intermediate. The crude intermediate (156 mg) was dissolved in EtOAc (6 mL) and 10% Pd/C (16 mg) was added and the mixture was stirred under a hydrogen balloon overnight. The reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. Purification by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm, 20–100% aqueous methanol over 15 minutes containing 0.1% TFA, 20 mL/min, monitoring at 220 nm) gave 160.3 mg (72%) of compound 425B as an off-white solid.

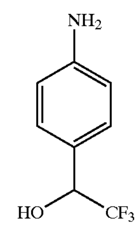
HPLC: 99% at 3.23 min (retention time) (Phenomenex-prime S5-C18 column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z [M+H]=512.19.

EXAMPLE 426

(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-[4-(2,2,2-trifluoro-1-hydroxyethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione (426B)



A. 1-(4-Amino-phenyl)-2,2,2-trifluoro-ethanol (426A)



Compound 426A was made according to the procedure described in Stewart, R. et al. *Can. J. Chem.* 58, 2491–2496 (1980). $NaBH_4$ (47 mg, 1.235 mmol) was added to a solution of p-aminotrifluoroacetophenone (155.7 mg, 0.823 mmol, synthesized as described by Klabunde, K. J. et al. *J. Org. Chem.* 35, 1711–1712 (1970)) in isopropanol (3 mL) at rt. After 30 min the reaction was quenched with phosphate buffer (pH 7.2), diluted with H_2O and extracted with EtOAc (2×10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give 154 mg (98%) of compound 426A as a tan solid. The material was used directly in the next step without purification. HPLC: 99% at 0.42 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z [M+H]=192.13.

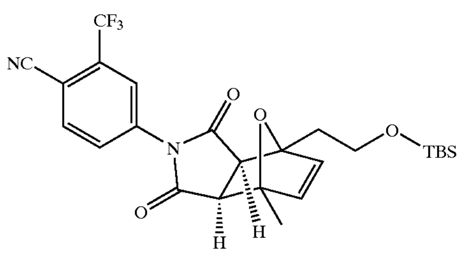
B. (3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-[4-(2,2,2-trifluoro-1-hydroxyethyl)phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione (426B)

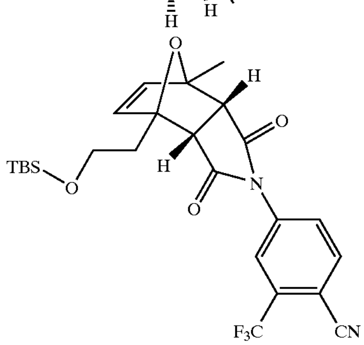
A mixture of compound 426A (75.3 mg, 0.394), compound 20A (51.5 mg, 0.262 mmol), triethylamine (0.15 mL) and $MgSO_4$ (50 mg) in toluene (1 mL) was heated in a sealed tube to 135° C. for 15 hr. The mixture was filtered and concentrated under reduced pressure. Purification by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm, 20–100% aqueous methanol over 15 minutes containing 0.1% TFA, 20 mL/min, monitoring at 220 nm) gave 63.1 mg

(65%) of compound 426B as a white solid. HPLC: 98% at 2.49 min (retention time) (Phenomenex-prime S5-C18 column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z [M+H]=370.16.

EXAMPLE 427

(3aα,4β,7β,7aα)-4-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-1,3,3a,4,7,7a-hexahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile & (3a α,4α,7α,7aα)-4-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-1,3,3a,4,7,7a-hexahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (427i & 427ii)

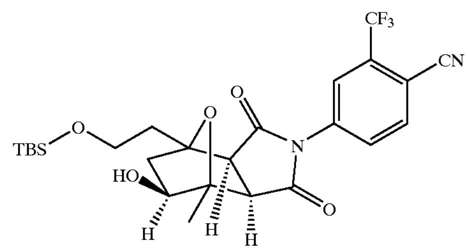


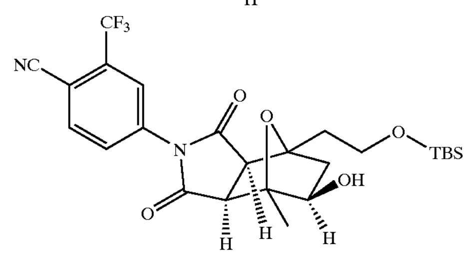


Compound 204A (2.00 g, 8.50 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (1.50 g, 5.60 mmol) were mixed in benzene (5.0 mL) and heated at 60° C. for 14 h, then cooled to 25° C. The solvent was removed at 40° C. under vacuum for 1 h to give the crude material which was purified by flash chromatography on SiO_2 eluting with 0.5% $EtOAc/CH_2Cl_2$ to give 2.0 g of compound 427i and 1.3 g of compound 427ii, both as light brown solids. Compound 427i: HPLC: 95% at 4.200 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 507.1 $[M+H]^+$. Compound 427ii: HPLC: 0.95% at 4.20 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 507.1 $[M+H]^+$.

EXAMPLE 428

[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile & [3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (428i & 428ii)





Compound 427i (1.40 g, 2.77 mmol) and $RhCl(PPh_3)_3$ (0.128 g, 0.14 mmol) were mixed in a flask. The flask was then evacuated and filled with argon three times, followed by the syringe addition of THF (3.0 mL). Once all particulates were dissolved, catecholborane (0.59 mL, 5.54 mmol) was added dropwise. The reaction mixture was stirred at 25° C. under argon for 30 min, then cooled to 0° C. Phosphate buffer (pH 7, 20 mL) was added, followed by EtOH (10 mL), 30% H_2O_2/H_2O (2 mL). The reaction mixture was stirred at 0° C. for 3 h, then extracted with dichloromethane (3×25 mL). The combined organic layers were washed with 1 N NaOH (25 mL), 10% Na_2SO_3 (25 mL) and brine (25 mL). The crude material was then concentrated in vacuo and purified by flash chromatography on SiO_2 eluting with 2% $EtOAc/CH_2Cl_2$ to 10% $EtOAc/CH_2Cl_2$ to give 0.63 g of a racemic mixture of compounds 428i & 428ii as a light yellow solid. HPLC: 99% at 3.867 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 525.1 [M+H].

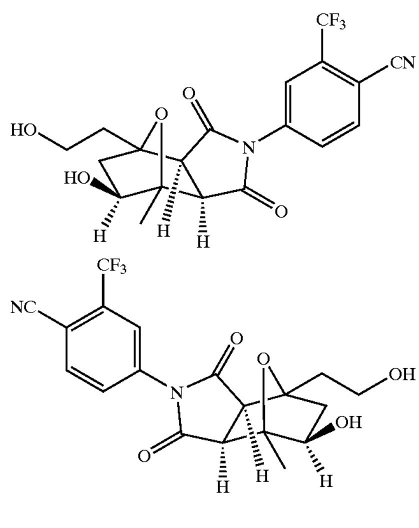
The racemic mixture of compounds 428i & 428ii was separated by normal phase preparative chiral HPLC using a Chiracel OD column (5 cm×50 cm), eluting with 13% solvent B (EtOH) in solvent A (hexanes), flow rate: 50 mL/min. Compound 428i eluted from 34 min to 38 min and compound 428ii eluted from 44 min to 49 min. Enantiomeric excess was determined by chiral HPLC. Compound 428i: >99% ee (12.576 min (retention time) (Chiralcel OJ column 4.6×250 mm eluting with isocratic 85% heptane 15%

MeOH/ethanol (1:1), 1 mL/min, monitoring at 220 nm, 40° C.). Compound 428ii: 99% ee (18.133 min (retention time) (Chiralcel OJ column 4.6×250 mm eluting with isocratic 85% heptane/15% MeOH/ethanol (1:1), 1 mL/min, monitoring at 220 nm, 40° C.).

The absolute configurations for compounds 428i & 428ii were not established. For simplicity in nomenclature, compound 428i is designated herein as having an "R" configuration and compound 428ii as having an "S" configuration. Enantiomerically pure products derived from compound 428i are designated herein as having a "R" configuration and enantiomerically pure products derived from compound 428ii are designated herein as having an "S" configuration.

EXAMPLE 429

[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-7-(2-hydroxyethyl)-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile & [3aS-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-7-(2-hydroxyethyl)-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (429i & 429ii)

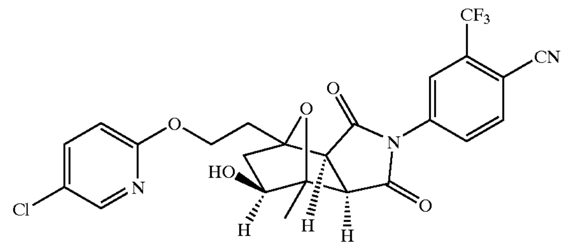


Compound 428i (180 mg, 0.34 mmol) was dissolved in 2% HCl/EtOH (5.0 mL). After 30 min, saturated $NaHCO_3$ was added and the aqueous layer was extracted with dichloromethane (20 mL×3), washed with brine and dried over Na_2SO_4 to give 135 mg of compound 429i as a white solid. HPLC: 99% at 2.257 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 411.1 $[M+H]^+$.

The above procedure was repeated with compound 428ii to yield the desired diol compound 429ii in similar yield.

EXAMPLE 430

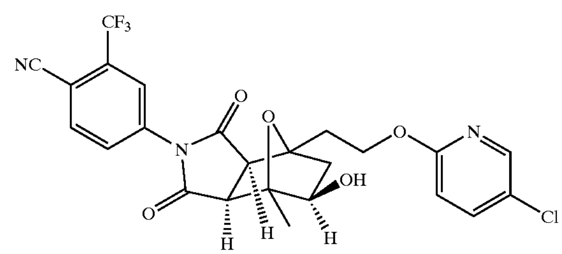
[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (430)



Triphenylphosphine (0.026 g, 0.098 mmol) and DBAD (0.023 g, 0.098 mmol) were mixed in THF (0.5 mL). After allowing the previous mixture to react for 15 min, 2-hydroxy-6-chloropyridine (0.016 g, 0.100 mmol) was added, the mixture was allowed to stir for 10 min and compound 429i (0.020 g, 0.049 mmol) was added. The reaction mixture was stirred at 25° C. for 2 h and then the crude material was purified by preparative TLC, eluting with 10% acetone/$CHCl_3$ to give 0.014 g of compound 0.430 as a light brown solid. HPLC: 100% at 3.370 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 522.08 $[M+H]^+$.

EXAMPLE 431

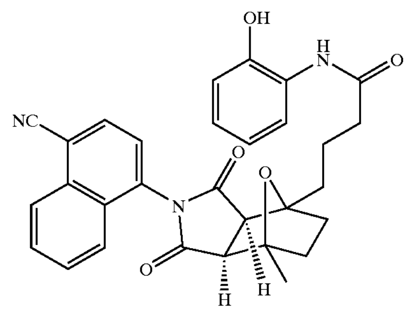
[3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (431)



Triphenylphosphine (0.026 g, 0.098 mmol) and DBAD (0.023 g, 0.098 mmol) were mixed in THF (0.5 mL). After allowing the previous mixture to react for 15 min, 2-hydroxy-6-chloropyridine (0.016 g, 0.100 mmol) was added, the mixture was allowed to stir for 10 mm and compound 429ii (0.020 g, 0.049 mmol) was added. The reaction mixture was stirred at 25° C. for 2 h and then the crude material was purified by preparative TLC, elating with 10% acetone/$CHCl_3$ to give 0.015 g of compound 431 as a light brown solid. HPLC: 100% at 3.370 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0;1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 522.08 $[M+H]^+$.

EXAMPLE 432

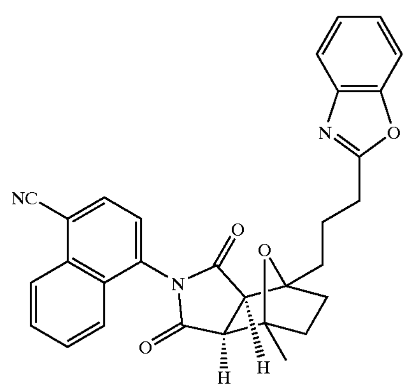
(3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthalenyl) octahydro-N-(2-hydroxyphenyl)-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindole-4-butanamide (432)



Compound 262 (0.100 g, 0.239 mmol) was dissolved in DMF (anhydrous 1.5 mL), BOP (0.211 g, 0.478 mmol) was added followed by 2-aminophenol (0.052 g, 0.478 mmol) and N-methyl morpholine (0.052 mL, 0.478 mmol). The reaction mixture was stirred at 25° C. under argon for 3 h, then the crude material was purified by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm, 20–100% aqueous methanol over 15 minutes containing 0.1% TFA, 20 mL/min, monitoring at 220 nm) to give 0.060 g of compound 432 as a light brown solid. HPLC: 100% at 3.037 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 510.34 $[M+H]^+$.

EXAMPLE 433

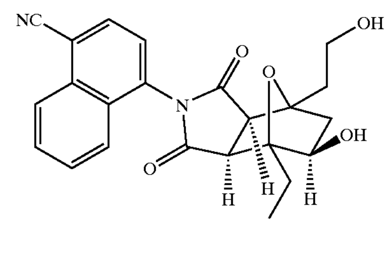
(3aα,4β,7β,7aα)-4-[4-[3-(2-Benzoxazolyl)propyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (433)



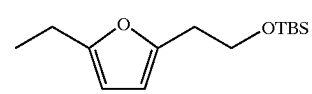
Triphenylphosphine (0.031 g, 0.118 mmol) and DBAD (0.027 g, 0.118 mmol) were mixed in THF (0.5 mL). After allowing the previous mixture to react for 15 min, compound 432 (0.030 g, 0.059 mmol) was added. The reaction mixture was stirred at 25° C. for 2 h and then the crude material was purified by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm, 20–100% aqueous methanol over 15 minutes containing 0.1% TFA, 20 mL/min, monitoring at 220 nm) to give 0.018 g of compound 433 as a light brown solid. HPLC: 100% at 3.357 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 492.37 $[M+H]^+$.

EXAMPLE 434

(3aα,4β,5β,7β,7aα)-4-[4-Ethyloctahydro-5-hydroxy-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (434C)

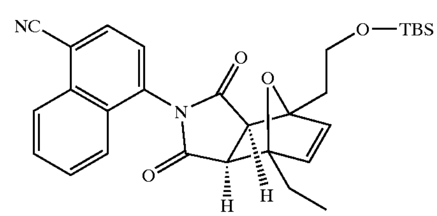


A. tert-Butyl-[2-(5-ethyl-furan-2-yl)-ethoxy]-dimethyl-silane (434A)



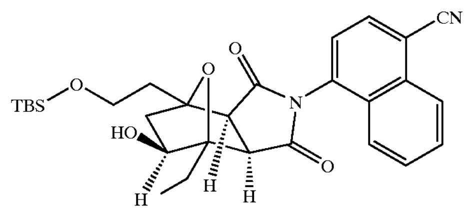
Imidazole (255 mg, 3.75 mmol) and TBSCl (414 mg, 2.75 mmol) were added to the solution of 245A (350 mg, 2.5 mmol) in DMF (4 mL). The mixture was stirred at rt for 15 hr and then 100 mg (0.66 mmol) of additional TBSCl was added to drive the reaction to completion. After stirring for an additional hour, the reaction mixture was diluted with diethylether (100 mL) and washed with water (20 mL), 1 N HCl (20 mL), water (20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give 509 mg of compound 434A (80.3%) as a yellow oil.

B. (3aα,4β,7β,7aα)-4-[4-[2-[[(1,1-Dimethylethyl)-dimethylsilyl]oxy]ethyl]-4-ethyl-1,3,3a,4,7,7a-hexahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (434B)



A solution of compound 434A (509 mg, 2.00 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-1-yl)-1-naphthalenecarbonitrile (498 mg, 2.00 mmol) in benzene (2 mL) was heated at 60° C. for 18 h. The reaction mixture was concentrated under reduced pressure to give 992 mg (99%) of crude compound 434B, which was used directly in the next step without further purification.

C. (3aα,4β,5β,7β,7aα)-4-[7-[2-[[(1,1-Dimethylethyl)-dimethylsilyl]oxy]ethyl]-4-ethyloctahydro-5-hydroxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (434C)



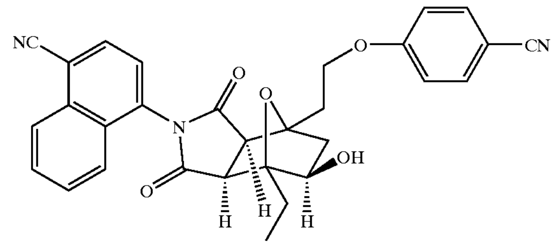
A mixture of compound 434B (992 mg, 1.98 mmol) and $RhCl_2(PPh_3)_3$ (183 mg, 0.198 mmol) was evacuated and filled with argon (3×). THF (20 mL) was added and once all particulates had dissolved, catecholborane (0.42 mL, 3.96 mmol) was slowly added dropwise. When the formation of product ceased, as was determined by HPLC, the reaction mixture was cooled to 0° C. and quenched with phosphate buffer (34 mL, pH 7.2) followed by the addition of EtOH (19 mL) and 30% H_2O_2 (2.9 mL). After 2 h, additional phosphate buffer (6.8 mL, pH 7.2), EtOH (3.8 mL) and H_2O_2 (0.6 mL) were added. The reaction mixture was stirred at rt for 3 h. Once the boronate intermediate was consumed, the mixture was extracted with CH_2Cl_2 (300 mL) and the combined organic layers were washed with 1 N NaOH, 10% aq. $NaHSO_3$ and brine and then dried over Na_2SO_4. Purification by flash chromatography on silica gel eluting with 10% $MeOH/CH_2Cl_2$ gave 75 mg (9.3%) of compound 434C as a gray solid. HPLC conditions: 97% at 2.43 min (retention time) (Phenomenex-prime S5-C18 column 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm). MS (ES): m/z 407.18 $[M+H]^+$.

D. (3aα,4β,5β,7β,7aα)-4-[4-Ethyloctahydro-5-hydroxy-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (434D)

Compound 434C (24 mg, 0.046 mmol) was dissolved in 2% conc. HCl/EtOH (0.8 mL) and the mixture was stirred at rt for 20 min. Cold sat. $NaHCO_3$ was added to the mixture until the solution was basic (pH 8). The reaction was extracted with EtOAc (3×2 mL) and the combined organic layers were washed with brine (2×5 mL) and dried over anhydrous sodium sulfate. Concentration in vacuo gave 14 mg (75%) of compound 434D as a white solid. HPLC: 95% at 2.40 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, monitoring at 220 nm).

EXAMPLE 435

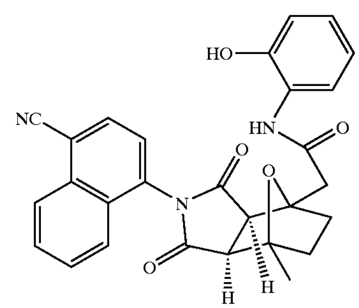
(3aα,4β,5β,7β,7aα)-4-[7-[2-(4-Cyanophenoxy)ethyl]-4-ethyloctahydro-5-hydroxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (435)



DBAD (39.6 mg, 0.172 mmol) was added to a solution of PPh_3 (45.1 mg, 0.172 mmol) in THF (0.8 mL). After stirring for 10 min, 4-cyanophenol (20.5 mg, 0.172 mmol) was added and the reaction mixture was stirred for an additional 5 min. Compound 434C (25.0 mg, 0.062 mmol) was added and the mixture was stirred at rt for 2 h. The reaction was concentrated under reduced pressure. Purification by Prep TLC eluting with 10% acetone/$CHCl_3$ gave 18.1 mg (0.036 mmol, 57.6%) of compound 435. HPLC conditions: 96% at 3.15 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm). MS (ES): m/z 508.14 $[M+H]^+$.

EXAMPLE 436

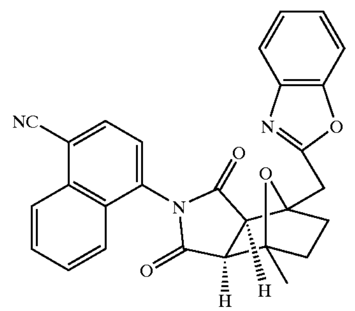
(3aα,4β,7β,7aα)-2-(4-Cyano-1-naphthyalenyl)octahydro-N-(2-hydroxyphenyl)-7-methyl-1,3-dioxo-4,7-expoxy-4H-isoindole-4-ethanamide (436)



Compound 234B (0.100 g, 0.256 mmol) was dissolved in DMF (anhydrous, 1.5 mL), BOP (0.225 g, 0.51 mmol) was added followed by 2-aminophenol (0.056 g, 0.51 mmol) and N-methyl morpholine (0.056 mL, 0.51 mmol). The reaction mixture was stirred at 25° C. under argon for 3 h, then the crude material was purified by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm, 20–100% aqueous methanol over 15 minutes containing 0.1% TFA, 20 mL/min, monitoring at 220 nm) to give 0.078 g of compound 436 as a light brown solid. HPLC: 100% at 3.037 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 482.34 $[M+H]^+$.

EXAMPLE 437

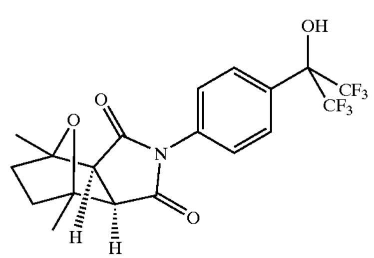
(3aα,4β,7β,7aα)-4-[4-(2-Benzoxazolylmethyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (437)



Triphenylphosphine (0.082 g, 0.312 mmol) and DBAD (0.072 g, 0.312 mmol) were mixed in THF (0.5 mL). After allowing the previous mixture to react for 15 min, compound 436 (0.075 g, 0.156 mmol) was added. The reaction mixture was stirred at 25° C. for 2 h and then the crude material was purified by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm, 20–100% aqueous methanol over 15 minutes containing 0.1% TFA, 20 mL/min, monitoring at 220 nm) to give 0.052 g of compound 437 as a light brown solid. HPLC: 100% at 3.443 min (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 464.18. $[M+H]^+$.

EXAMPLE 438

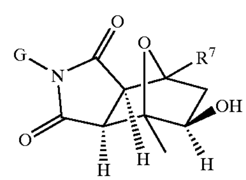
(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione (438)



A mixture of 2-(4'-aminophenyl)-1,1,1,3,3,3-hexafluoro-2-propanol (95.7 mg, 0.369), compound 20A (48.3 mg, 0.246 mmol), triethylamine (0.15 mL) and $MgSO_4$ (50 mg) in toluene (1 mL) was heated in a sealed tube to 135° C. overnight. The mixture was filtered and concentrated under reduced pressure. Purification by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm, 20–100% aqueous methanol over 15 minutes containing 0.1% TFA, 20 mL/min, monitoring at 220 nm) gave 44.0 mg (41%) of compound 438 as a white solid. HPLC: 99% at 3.10 min (retention time) (Phenomenex-prime S5-C18 column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z [M+H]=438.14.

EXAMPLES 439 TO 454

Additional compounds of the present invention were prepared by procedures analogous to those described above. The compounds of Examples 439 to 454 have the following structure (L is a bond):



where G, R^7, the compound name, retention time, molecular mass, and the procedure employed, are set forth in Table 9. The absolute configuration for the following compounds was not determined. For simplicity in nomenclature, compound 243Di is designated herein as having an “S” configuration and compound 243Dii as having an “R” configuration. Enantiomerically pure products derived from compound 243Di are designated herein as having an “S” configuration and enantiomerically pure products derived from compound 243Dii are designated herein as having an “R” configuration. Similarly, compound 428i is designated herein as having an “S” configuration and compound 428ii as having an “R” configuration. Enantiomerically pure products derived from compound 428i are designated herein as having an “S” configuration and enantiomerically pure products derived from compound 428ii are designated herein as having an “R” configuration.

The chromatography techniques used to determine the compound retention times of Table 9 are as follows: LCMS=YMC S5 ODS column, 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 4 minutes containing 0.1% TFA; 4 mL/min, monitoring at 220 nm. LCMS*=YMC S5 ODS column, 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 2 minutes containing 0.1% TFA; 4 mL/min, monitoring at 220 nm. LC=YMC S5 ODS column 4.6×50 mm eluting with 10–90% $MeOH/H_2O$ over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm. The molecular mass of the compounds listed in Table 9 were determined by MS (ES) by the formula m/z.

TABLE 9

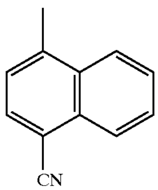
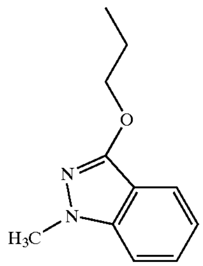
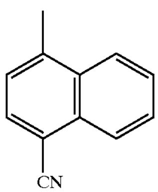
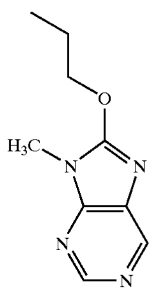
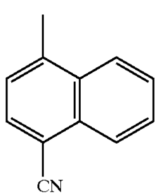
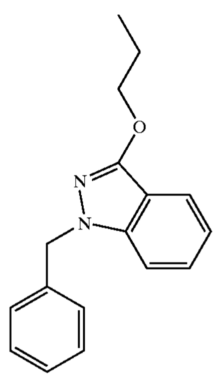
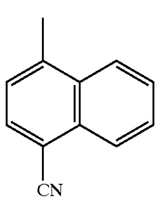
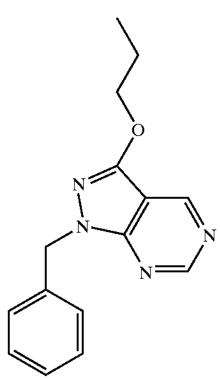
Ex. No	G	R⁷	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
439			[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-7-[2-[(1-methyl-1H-indazol-3-yl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile	3.33 LC 523.3 $[M + H]^+$	251, 253
440			[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-7-[2-[(9-methyl-9H-purin-8-yl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile	2.34 LC 525.2 $[M + H]^+$	251, 253
441			[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[[1-(phenylmethyl)-1H-indazol-3-yl]oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile	3.73 LC	251, 253
442			[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[[1-(phenylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl]oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile	3.37 LC	251, 253

TABLE 9-continued

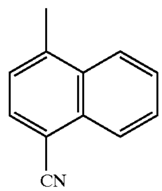
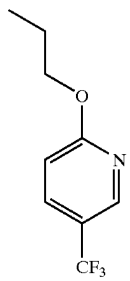
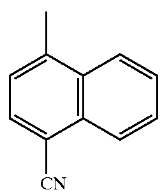
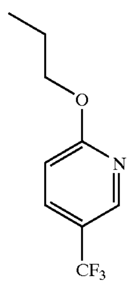
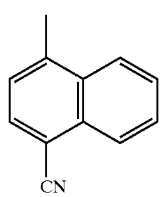
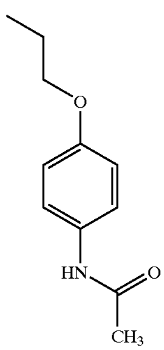
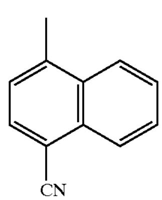
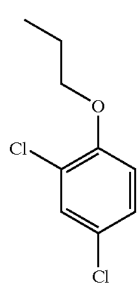
Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
443			[3aS-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[[5-(trifluoromethyl)-2-pyridinyl]oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile	3.45 LC 538.23 $[M + H]^+$	243Di, 244i
444			[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[[5-(trifluoromethyl)-2-pyridinyl]oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile	3.46 LC 538.24 $[M + H]^+$	243Dii, 244ii
445			[3aR-(3aα,4β,5β,7β,7aα)]-N-[4-[2-[2-(4-Cyano-1-naphthalenyl)octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-7H-isoindol-7-yl]ethoxy]phenyl]acetamide	2.747 LC 526.28 $[M + H]^+$	243Dii, 244ii
446			[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(2,4-Dichlorophenoxy)-ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile	3.71 LC 537.17 $[M + H]^+$	243Dii, 244ii

TABLE 9-continued

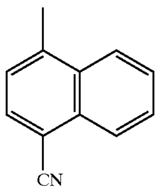
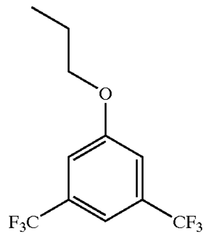
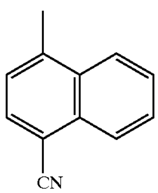
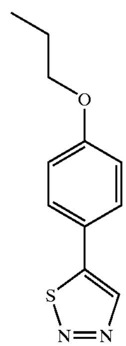
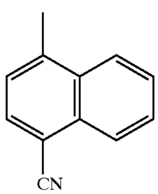
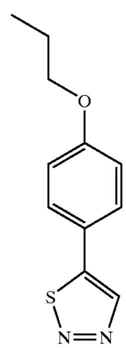
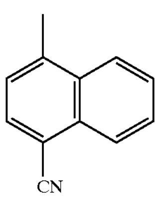
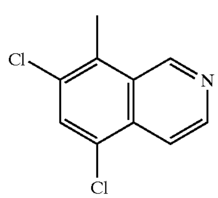
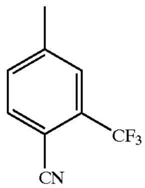
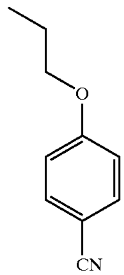
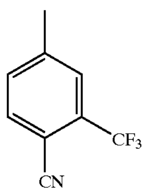
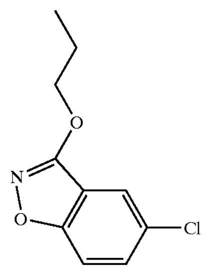
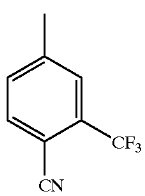
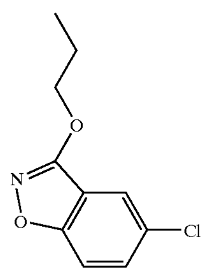
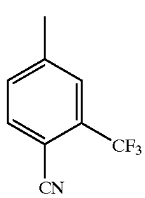
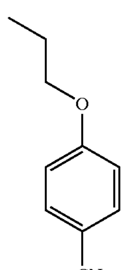
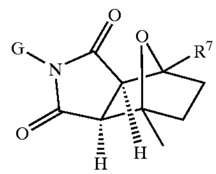
Ex. No	G	R7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
447			[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[3,5-Bis(trifluoromethyl)phenoxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.89 LC 605.25 $[M + H]^+$	243Dii, 244ii
448			[3aS-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[4-(1,2,3-thiadiazol-5-yl)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.14 LC 553.1 $[M + H]^+$	243Di, 244i
449			[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[4-(1,2,3-thiadiazol-5-yl)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.15 LC 553.23 $[M + H]^+$	243Dii, 244ii
450			[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5,7-Dichloro-8-quinolinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	3.70 LC 588.26 $[M + H]^+$	243Dii, 244ii

TABLE 9-continued

Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
451			[3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(4-Cyanophenoxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile	3.087 LC 512.13 $[M + H]^+$	431
452			[3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-1,2-benzisoxazol-3-yl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile	3.563 LC 562.08 $[M + H]^+$	431
453			[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-1,2-benzisoxazol-3-yl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile	3.57 LC 562.08 $[M + H]^+$	430
454			[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(4-Cyanophenoxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile	3.087 LC 512.08 $[M + H]^+$	430

EXAMPLES 455 TO 457

Additional compounds of the present invention were prepared by procedures analogous to those described above. The compounds of Examples 455 to 457 have the following structure (L is a bond):

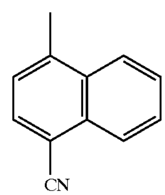
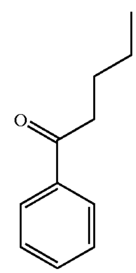
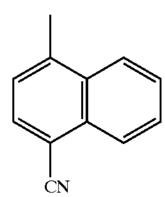
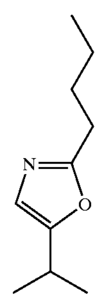
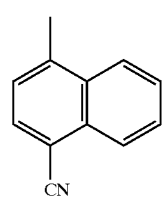
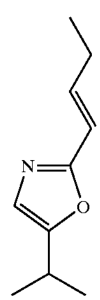


where G, R^7, the compound name, retention time, molecular mass, and the procedure employed, are set forth in Table 10. The absolute configuration for the following compounds was not determined. For simplicity in nomenclature, compound 238i is designated

herein as having an "R" configuration and compound 238ii as having an "S" configuration. Enantiomerically pure products derived from compound 238i are designated herein as having an "R" configuration and enantiomerically pure products derived from compound 238ii are designated herein as having an "S" configuration.

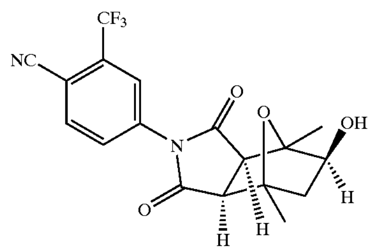
The chromatography techniques used to determine the compound retention times of Table 10 are as follows: LCMS=YMC S5 ODS column, 4.6×50 mm eluting with 10–90% MeOH/H_2O over 4 minutes containing 0.1% TFA; 4 mL/min, monitoring at 220 nm. LCMS*=YMC S5 ODS column, 4.6×50 mm eluting with 10–90% MeOH/H_2O over 2 minutes containing 0.1% TFA; 4 mL/min, monitoring at 220 nm. LC=YMC S5 ODS column 4.6×50 mm eluting with 10–90% MeOH/H_2O over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm. The molecular mass of the compounds listed in Table 10 were determined by MS (ES) by the formula m/z.

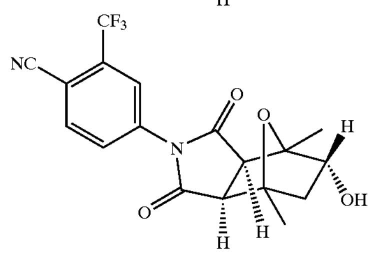
TABLE 10

Ex. No	G	R^7	Compound Name	Retention Time Min./ Molecular Mass	Procedure of Ex.
455			(3aα,4β,5β,7β,7aα)-4-[Octahydro-4-methyl-1,3-dioxo-7-(4-oxo-4-phenylbutyl)-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile	3.53 LC 479.35 $[M + H]^+$	265, 266
456			(3aα,4β,5β,7β,7aα)-4-[Octahydro-4-methyl-7-[3-[5-(1-methylethyl)-2-oxazolyl]propyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile	3.547 LC 484.28 $[M + H]^+$	248, 249
457			[3aα,4β,5β,7β,7aα(E)]-4-[Octahydro-4-methyl-7-[3-[5-(1-methylethyl)-2-oxazolyl]-2-propenyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalene-carbonitrile	3.66 LC 482.28 $[M + H]^+$	248, 249

EXAMPLE 458

(3aα,4β,5β,7β,7aα)-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile & (3aα,4β,5α,7β,7aα)-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (221B & 222D)





Compound 20B was converted to compounds 221B and 222D (also synthesized as compounds 221B and 222D) by biotransformation.

Compound 20B was hydroxylated by *Amycolatopsis orientalis* (ATCC 43491). A 1 mL culture from a frozen vial was used to inoculate 100 mL medium in a 500 mL portion Erlenmeyer flask and the flask was incubated at 28° C., at 200 rpm for 3 days. A 10 mL portion of this culture was used to inoculate 100 mL medium in a 500 mL Erlenmeyer flask and the flask was incubated at 28° C., at 200 rpm for 1 day. 10 mL portions of the 1-day culture were distributed to each of three 5.0 mL flasks. Compound 20B (3 mg in 0.1 mL methanol) was added to each culture and the incubations were continued for 3 days. The culture broth in each flask was extracted with 20 mL ethyl acetate, and the pooled ethyl acetate extracts were evaporated to dryness at 40° C. under a nitrogen stream. The residue was dissolved in 1.2 mL methanol and analyzed by HPLC, LC/MS and LC/NMR. The solution contained 2.5 mg of remaining Compound 20B, 1.6 mg of compound 221B, and 1.3 mg of compound 222D. MS and NMR analyses were in agreement with the structures shown above.

Medium: 0.5% toasted nutrisoy, 2% glucose, 0.5% yeast extract, 0.5% K_2HPO_4, 0.5% NaCl, adjusted to pH 7 with HCl (R. V. Smith and J. P. Rosazza, Arch. Biochem. Biophys., 161, 551–558 (1974)

HPLC Analysis

Column: Phenomenex Luna C18, 150×2 mm, 5μ

mobile phase:

solvent A: 95% 20 nM ammonium acetate pH 5.1, 5% acetonitrile

solvent B: 95% acetonitrile, 5% 20 mM ammonium acetate pH 5.1 linear gradient going from 100% solvent A to 5% solvent A in 25 minutes followed by equilibration at 100% solvent A for 8 minutes.

temperature: 40° C.

detection: 250 nm

injection volume: 1 μL

retention times: compound 20B, 20.8 min; compound 221B, 16.5 min; compound 222D, 17.8 min

HPLC Conditions

Chiral HPLC conditions were employed for the separation of enantiomers and achiral HPLC conditions were employed for the separation of diastereomers of the hydroxylated analogs of compound 20B (i.e., compounds 221B and 222D and compounds 254i and 254ii)

Two methods were used under chiral HPLC conditions, reverse phase (RP) for chiral analysis of biotransformation products in biological samples and normal phase (NP) for non-biological samples.

Chiral RP-HPLC Condition	
Column:	CHIRALPAK AD-R 4.6 × 250 mm, 10 μ
Temperature:	40° C.
Injection Volume:	5 or 20 μL
Mobile Phase:	A: MeCN B: H_2O Isocratic, 30% of A, 18 min.
Flow Rate:	1 mL/min.
UV Detection:	242 nm
Chiral NP-HPLC Condition	
Column:	CHIRALPAK AD 4.6 × 250 mm, 10 μ
Temperature:	25° C.
Injection Volume:	5 or 20 μL
Mobile Phase:	A: Heptane B: MeOH/Ethanol (1:1) Isocratic, 80% of A, 20 min.
Flow Rate:	1 mL/min.
UV Detection:	242 nm

Under these conditions compounds 254i and 254ii had retention times of 8.5 minutes and 9.85 minutes, respectively.

Reverse phase HPLC was employed for the separation of the diastereomeric compounds 221B and 222D:

Mobile Phase:

Solvent A: 95% 20 mM ammonium acetate pH 5.1, 5% acetonitrile

Solvent B: 95% acetonitrile, 5% 20 mM ammonium acetate pH 5.1

Gradient:

Linear gradient going from 100% solvent A to 5% solvent A in 25 minutes followed by equilibration at 100% solvent A for 8 minutes. Total run time of 36 minutes.

Flow Rate:

0.2 mL/min

Column:

Phenomenex Luna 5 micron C_{18} 150×2.0 mm id

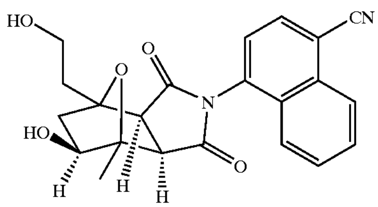
Detection:

UV detection at 242 nm

Under these conditions, compounds 221B and 222D had retention times of 18.983 min and 20.362 min, respectively.

EXAMPLE 459

(3aα,4β,5β,7β,7aα)-4-[Octahydro-5-hydroxy-7-(2-hydroxyethyl)-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (459)



Compounds 223A and 331 were converted to compound 459 by biotransformation.

Microbial Hydroxylation of Compound 223A

1. Reaction

To a 500 mL flask containing 100 mL of the transformation medium was added one frozen vial (approximately 2 mL) of *Streptomyces griseus* ATCC 10137. The transformation medium was prepared as follows: to a 2 L plastic beaker was added 20 g of dextrose, 5.0 g of yeast extract, 5.0 g of soybean meal, 5.0 g of sodium chloride, 5.0 g of potassium phosphate (diabasic) and 1 L of deionized water, and the mixture was stirred at room temperature for 3 to 30 min. The pH of the mixture was then adjusted to 7.0 with 1 N HCl or 1 N NaOH. The resulting mixture was dispensed into 500 mL flasks (100 mL per flask). The flasks were covered with Bio/Wrap and autoclaved at 121° C. for 15 min. and cooled down to room temperature before use.

The culture was incubated at 28° C. and at 250 rpm for 24 hours. Ten mL of the resulting culture was transferred to a 50 mL flask, to which 1 mg of compound 223A in 0.2 mL ethanol was added. The flask was incubated at 28° C. and 250 rpm for 24 hours, and the reaction culture was extracted with EtOAc (10 mL). The EtOAc extract was dried under N_2 and the residue was dissolved in 1 mL of MeOH (reaction extract).

2. Product Analysis

HPLC:

10 μL of the reaction extract was injected into HPLC column (YMC ODS-AQ C-18 column, 150×6.0 mm i.d.). The column was eluted with 1 mM HCl in water/CH_3CN at 1.2 mL/min flow rate: 30 to 60% CH_3CN over 8 min, 60 to 85% CH_3CN over 0.5 min, 85% CH_3CN for 1 min, 85 to 30% CH_3CN over 0.5 min. The eluents were monitored at 300 nm. Two major peaks with about a 1 to 1 area ratio were observed, which had the same UV spectra as those of compounds 459 and 331, and had retention times of 4.55 min and 7.23 min, respectively, matching the retention times of authentic samples of compound 459 (4.53 min) and compound 331 (7.2 min).

LC/MS

The reaction extract: two major UV peaks.
Peak 1, Tr 4.68 min: 391 $[M+H]^+$, 343, 319, 303, 289
Peak 2, Tr 5.35 min: 375 $[M+H]^+$, 345

Authentic Samples

Compound 459, Tr 4.82 min: 391. $[M+H]^+$, 343, 319, 289
Compound 331, Tr 5.48 min: 375 $[M+H]^+$, 345

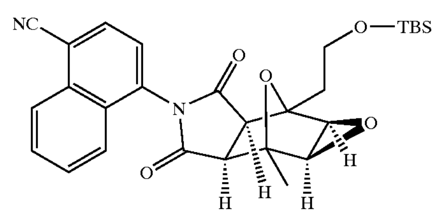
Microbial Hydroxylation of Compound 331

To a 500 mL flask containing 100 mL of the transformation medium was added one frozen vial (approximately 2 mL) of *Streptomyces griseus* ATCC 10137. The transformation medium was prepared as follows: to a 2 L plastic beaker was added 20 g of dextrose, 5.0 g of yeast extract, 5.0 g of soybean meal, 5.0 g of sodium chloride, 5.0 g of potassium phosphate (dibasic) and one L of deionized water, and the mixture was stirred at room temperature for 3 to 30 min. The pH of the mixture was then adjusted to 7.0 with 1 N HCl or 1 N NaOH. The resulting mixture was dispensed into 500 mL flasks (100 mL per flask). The flasks were covered with Bio/Wrap and autoclaved at 121° C. for 15 min. and cooled down to room temperature before use.

The culture was incubated at 28° C. and 250 rpm for 3 days. One mL of the resulting culture was added to a 500 mL flask containing 100 mL of the transformation medium and the flask was incubated at 28° C. and 250 rpm for 24 hours. Ten mL of the resulting culture was transferred to a 50 mL flask, to which 1 mg of compound 331 in 0.2 mL ethanol was added. The flask was incubated at 28° C. and 250 rpm for 23 hours. HPLC analysis showed that the peak area ratio of compound 459 to compound 331 in the reaction culture was about 1.1/1.

EXAMPLE 460

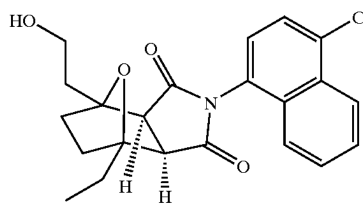
(1aα,2β,2aα,5aα,6βb,6aα)-4-[2-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-6-methyl-3,5-dioxo-2,6-epoxy-4H-oxireno[f]isoindol-4-yl]-1-naphthalenecarbonitrile (460)

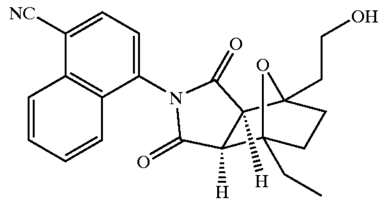


Compound 231A (2.00 g, 4.10 mmol) was dissolved in dicholomethane (40 mL) and cooled to 0° C. mCPBA (2.36 g, 8.20 mmol) was added. The reaction mixture was then warmed up to room temperature and stirred under argon for 18 hours, followed by the addition of 10% Na_2SO_3 (25 mL) and saturated $NaHCO_3$ (25 mL). After stirring for 20 minutes, the organic layer was separated and the aqueous layer was extracted with dicholomethane (3×50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give 2.0 g compound 460 as light yellow solid. HPLC: 99% at 4.00 min (retention time) (Phenomenex-prime S5-C18 column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z [M+H]=505.19.

EXAMPLE 461

[3aR-(3aα,4β,7β,7aα)]-4-[4-Ethyloctahydro-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile & [3aS-(3aα,4β,7β,7aα)]-4-[4-Ethyloctahydro-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitril (461i & 461ii)



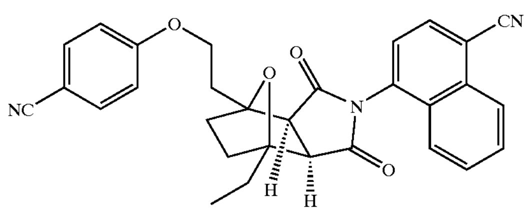


The racemic mixture of compounds 245C was separated by normal phase preparative chiral HPLC using a Chiracel AD column (5 cm×50 cm), eluting with 20% solvent B (50% MeOH/EtOH) in solvent A (Heptane), flow rate: 50 mL/min. Compound 461i eluted from 80 min to 100 min and compound 461ii eluted from 125 min to 150 min.

The absolute conformation for compounds 461i and 461ii was not determined. For simplicity in nomenclature, compound 461i is designated herein as having an "R" configuration and compound 461ii as having an "S" configuration. Enantiomerically pure products derived from compound 461i are designated herein as having an "R" configuration and enantiomerically pure products derived from compound 461ii are designated herein as having an "S" configuration.

EXAMPLE 462

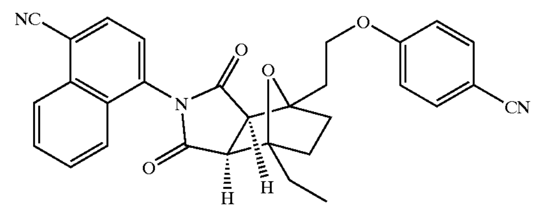
[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(4-Cyanophenoxy)ethyl]-7-ethyloctahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (462)



DBAD (29.5 mg, 0.128 mmol) was added to a solution of PPh_3 (33.6 mg, 0.128 mmol) in THF (0.5 mL). After stirring for 10 min, 4-cyanophenol (15.2 mg, 0.128 mmol) was added and the reaction mixture was stirred for an additional 5 min. Compound 461i (18.3 mg, 0.047 mmol) was added and the mixture was stirred at rt for 2 h. The reaction was concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 40% EtOAc/hexane gave 16.9 mg (0.034 mmol, 73.2%) of compound 462. HPLC conditions: 98% at 3.64 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm). MS (ES): m/z 492.23 $[M+H]^+$.

EXAMPLE 463

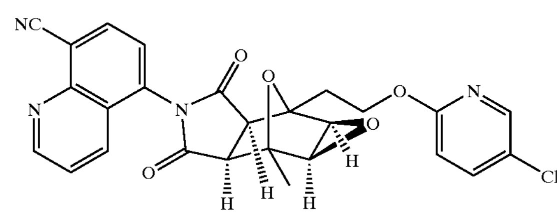
[3aS-(3aβ,4β,7β,7aα)]-4-[4-[2-(4-Cyanophenoxy)ethyl]-7-ethyloctahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (463)



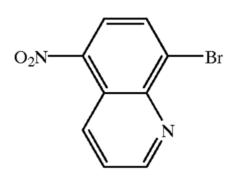
DBAD (29.5 mg, 0.128 mmol) was added to a solution of PPh_3 (33.6 mg, 0.128 mmol) in THF (0.5 mL). After stirring for 10 min, 4-cyanophenol (15.2 mg, 0.128 mmol) was added and the reaction mixture was stirred for an additional 5 min. Compound 461ii (18.3 mg, 0.047 mmol) was added and the mixture was stirred at rt for 2 h. The reaction was concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 40% EtOAc/hexane gave 18.1 mg (0.037 mmol, 78.4%) of compound 463. HPLC conditions: 97% at 3.63 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 minute gradient with 0.2% H_3PO_4, detecting at 220 nm). MS (ES): m/z 492.17 $[M+H]^+$.

EXAMPLE 464

(1aα,2β,2aα,5aα,6β,6aα)-5-[2-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-6-methyl-3,5-dioxo-2,6-epoxy-4H-oxireno[f]isoindol-4-yl]-8-quinolinecarbonitrile (464H)



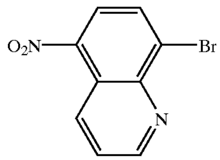
A. 8-Bromo-5-nitro-quinoline (464A)



8-Bromoquinoline (25.00 g, 120.2 mmol) was dissolved in sulfuric acid (82.5 mL) at rt and then cooled to 0° C. HNO_3 (fuming, 32.5 mL) was then slowly added over a 10 minute period. The reaction was then warmed to rt and then to 65° C. After 48 h at 65° C., the reaction was cooled to rt and poured onto 500 g of ice. This solution was extracted with methylene chloride (5×200 mL). The organic layers were washed once with brine and dried over anhydrous

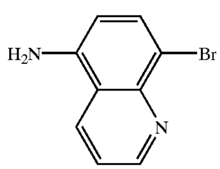
sodium sulfate. Concentration gave the crude compound 464A as a light yellow solid (28.6 g, 94%). HPLC: 98% at 2.717 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

B. 5-Nitro-quinoline-8-carbonitrile (464B)



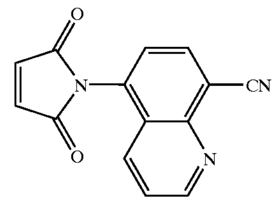
Compound 464A (15.0 g, 59.3 mmol) was dissolved in DMF (120 mL) and zinc cyanide (4.20 g, 35.9 mmol) was added. Bis(diphenylphosphino)ferrocene (3.00 g, 5.40 mmol) and tris(benzylidineacetone)dipalladium (3.00 g, 3.30 mmol) were then added and the reaction was heated to 100° C. for 1.5 h. The reaction was cooled to 22° C. and then poured into concentrated ammonium hydroxide (900 mL) resulting in an orange precipitate which was filtered and rinsed with cold water (1 L). The resulting precipitate was dissolved in methylene chloride, washed with brine (1×300 mL) and then dried over anhydrous sodium sulfate. Concentration in vacuo gave the crude material as an orange solid which was purified by flash chromatography on silica gel eluting with methylene chloride to give 6.01 g (51%) of compound 464B as a yellow solid. HPLC: 99% at 1.900 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

C. 5-Amino-quinoline-8-carbonitrile (464C)



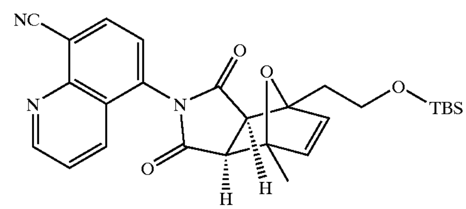
Compound 464B (6.00 g, 30.1 mmol) was dissolved in THF (150 mL) at reflux with mechanical stirring. EtOH (150 mL) was then added followed by aqueous ammonium chloride (2.4 g/225 mL water). The mixture was heated at 70° C. and then iron powder (325 mesh, 6.75 g, 120 mmol) was added with vigorous mechanical stirring. After 1 h, the reaction was cooled to 22° C. and filtered through Celite rinsing with methylene chloride. The filtrate was then concentrated to ~250 mL and the pH was adjusted to 10 by addition of 1N NaOH. The solution was then extracted with ethyl acetate (5×150 mL). The combined organic layers were washed once with brine (250 mL) and then dried over anhydrous magnesium sulfate. Concentration in vacuo gave 5.09 g (100%) of compound 464C as a yellow solid. HPLC: 99% at 1.143 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 170.16 $[M+H]^+$.

D. 5-(2,5-Dioxo-2,5-dihydro-pyrrol-1-yl)-quinoline-8-carbonitrile (464D)



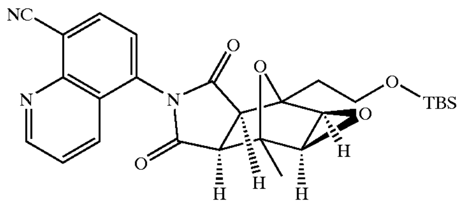
Compound 464C (7.00 g, 41.4 mmol) and maleic anhydride (6.00 g, 62.1 mmol) were combined in a sealed tube and THF (10 mL) was added. The reaction mixture was heated to 115° C. for 15 min then cooled to room temperature, resulting in the precipitation of the intermediate acid amide. The solid was filtered and rinsed with cold THF to give 11.0 g of the acid as a yellow solid. To the above acid amide was added Ac_2O (25 mL) in a sealed tube and the mixture was heated at 100° C. for 15 min then cooled to room temperature. The resulting solid was filtered and rinsed with cold diethyl ether to give 8.30 g (80%) of compound 464D as a yellow solid. HPLC: 97% at 1.783 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

E. (3aα,4β,7β,7aα)-5-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-1,3,3a,4,7,7a-hexahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (464E)



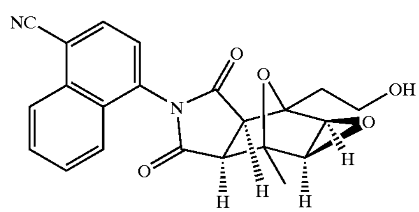
Compound 464D (6.00 g, 24.1 mmol) was dissolved in a mixture of benzene (80 mL) and acetone (20 mL) followed by addition of compound 204A (14.46 g, 60.15 mmol). The mixture was heated at 80° C. for 14 h and then cooled to 22° C. Concentration in vacuo at 40° C. followed by addition of acetone (40 mL) and concentration again at 40° C. The resulting yellow oil was purified by flash column chromatography on silica gel eluting with 0–10% acetone in chloroform to give 9.98 g (85%) of compound 464E as a yellow oil. Compound 464E was shown to be a single isomer by NMR spectroscopy. HPLC: 97% at 3.853 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 490.35 $[M+H]^+$.

F. (1aα,2β,2aα,5aα,6β,6aα)-5-[2-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-6-methyl-3,5-dioxo-2,6-epoxy-4H-oxireno[f]isoindol-4-yl]-8-quinolinecarbonitrile (464F)



To a solution of compound 464E (0.050 g, 0.10 mmol) in dichloromethane (2 mL) was added mCPBA (60% mixture, 0.063 g, 0.22 mmol). The reaction mixture was stirred at room temperature for 16 h and then additional dichloromethane (20 mL), saturated $NaHCO_3$ (10 mL) and 10% Na_2SO_3 (10 mL) were added. The mixture was stirred vigorously for 40 min, the organic layer was then separated, washed once with brine, and dried over Na_2SO_4. Concentration in vacuo gave 48 mg (96%) of compound 464F as a light-yellow solid. HPLC: 98% at 3.783 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 506.25 $[M+H]^+$.

G. (1aα,2β,2aα,5aα,6β,6aα)-5-[Octahydro-2-(2-hydroxyethyl)-6-methyl-3,5-dioxo-2,6-epoxy-4H-oxireno[f]isoindol-4-yl]-8-quinolinecarbonitrile (464G)



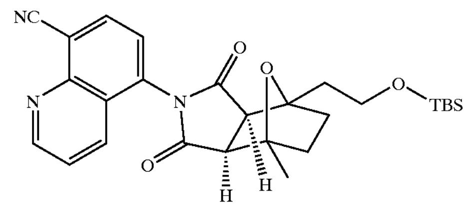
Compound 464F (1.30 g, 2.57 mmol) was dissolved in 2% conc. HCl/EtOH (50 mL). The reaction mixture was stirred at room temperature for 1 h and then saturated $NaHCO_3$ (50 mL) and dichloromethane (100 mL) were added. The organic layer was separated, washed once with brine and dried over Na_2SO_4. Concentration in vacuo gave 930 mg (93%) of compound 464G as a yellow solid. HPLC: 98% at 1.863 (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 392;20 $[M+H]^+$.

H. (1aα,2β,2aα,5aα,6β,6aα)-5-[2-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-6-methyl-3,5-dioxo-2,6-dioxo-2,6-epoxy-4H-oxireno[f]isoindol-4-yl]-8-quinolinecarbonitrile (464H)

Triphenylphosphine (25 mg, 0.096 mmol) and DBAD (22 mg, 0.096 mmol) were mixed in THF (0.5 mL) under argon. After 5 min, 5-chloro-2-pyridinol (13 mg, 0.096 mmol) was added. The reaction mixture was stirred at 22° C. for another 10 min, then compound 464G (25 mg, 0.064 mmol) was added. The reaction mixture was stirred at 22° C. under argon for 3 h, and then concentrated in vacuo. The crude material was purified by preparative TLC on silica gel eluting with 10% acetone in chloroform to give 11 mg (23%) of compound 464H as a white solid. HPLC: 100% at 3.177 (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 503.14 $[M+H]^+$.

EXAMPLE 465

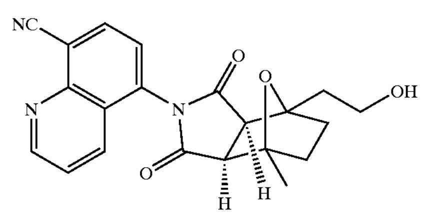
(3aα,4β,7β,7aα)-5-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (465)



Compound 464E (2.40 g, 4.91 mmol) was dissolved in ethyl acetate and Pd/C (10% Pd, 0.50 g) was added. Hydrogen was then introduced via a balloon. After 3 h, the reaction was purged with nitrogen and filtered through Celite, rinsing with ethyl acetate. Concentration in vacuo gave 2.39 g (99%) of compound 465 as a yellow oil. HPLC: 95% at 4.013 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 492.22 $[M+H]^+$.

EXAMPLE 466

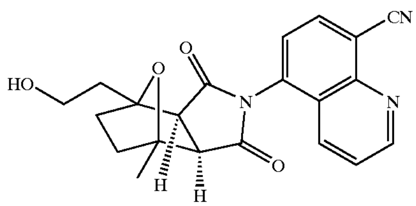
(3aα,4β,7β,7aα)-5-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (466)

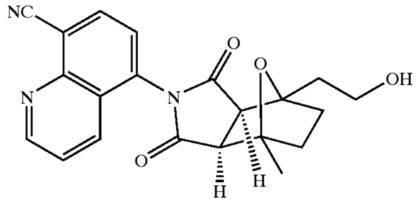


Compound 465 (1.40 g, 2.85 mmol) was dissolved in 2% conc. HCl/MeOH (20 mL) and stirred at 22° C. for 3 h. The reaction was then concentrated to ~5 mL volume and quenched with a minimum amount of sat. aq. sodium bicarbonate. This solution was then extracted with methylene chloride (3×30 mL) and the combined organic layers were dried over anhydrous sodium sulfate. Concentration in vacuo gave 0.893 g (93%) of compound 466 as a yellow solid. This material was taken on without further purification. HPLC: 98% at 2.140 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 378.25 $[M+H]^+$.

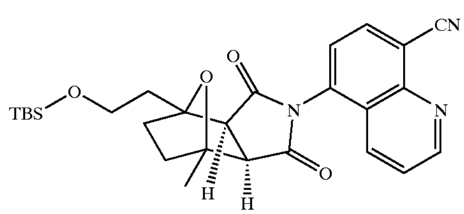
EXAMPLE 467

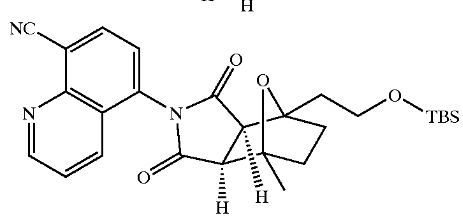
[3aR-(3aα,4β,7β,7aα)]-5-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (467Bi) & [3aS-(3aα,4β,7β,7aα)]-5-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (467Bii)





A. [3aR-(3aα,4β,7β,7aα)]-5-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (467Ai) & [3aS-(3aα,4β,7β,7aα)]-5-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (467Aii)





Compounds 465 was separated into its individual antipodes by normal phase preparative chiral HPLC. A Chiralcel OD column (50×500 mm) was used with a flow rate of 50 mL/min (16% EtOH/hexanes) monitoring at 220 nm. The faster eluting antipode compound 467Ai had a retention time of 40.85 min (>99% ee) and the slower antipode compound 467Aii had a retention time of 62.81 min (>99% ee). Both antipodes were isolated as white solids after separation. The absolute conformation for compounds 467Ai & 467Aii was not established. For simplicity in nomenclature, compound 467Ai is designated herein as having an "R" configuration and compound 467Aii as having an "S" configuration. Enantiomerically pure products derived from compound 467Ai are designated herein as having a "R" configuration and enantiomerically pure products derived from compound 467Aii are designated herein as having an "S" configuration.

B. [3aR-(3aα,4β,7β,7aα)]-5-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (467Bi) & [3aS-(3aα,4β,7β,7aα)]-5-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (467Bii)

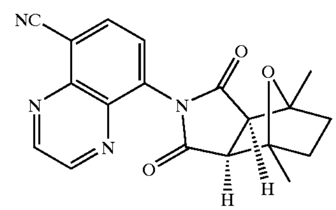
Both antipodes were deprotected as described in example 464G to give the corresponding alcohols, compounds 467Bi and 467Bii as white solids:

Compound 467Bi: HPLC: 98% at 2.110 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 378.21 $[M+H]^+$.

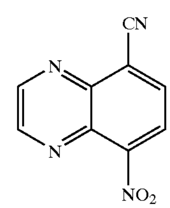
Compound 467Bii: HPLC: 98% at 2.117 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 378.20 $[M+H]^+$.

EXAMPLE 468

(3aα,4β,7β,7aα)-8-[Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-5-quinoxalinecarbonitrile (468C)

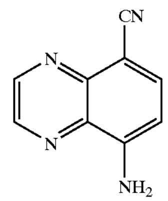


A. 8-Nitro-quinoxaline-5-carbonitrile (468A)



2,3-Diamino-4-nitro-benzonitrile (0.050 g, 0.28 mmol, as prepared in WO-98/32439) was added to solution of glyoxal (40% in water, 0.032 mL, 0.28 mmol) in acetic acid (0.75 mL) and stirred at 22° C. for 3 h. The reaction was cooled to 0° C. and water (2.0 mL) was added and the pH was adjusted to 9.0 by addition of ammonium hydroxide which caused the product to precipitate. The mixture was then filtered and rinsed with cold water. Drying in vacuo gave 0.039 g (70%) of compound 468A as an orange solid. HPLC: 100% at 2.037 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

B. 8-Amino-quinoxaline-5-carbonitrile (468B)



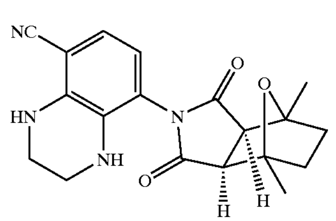
Compound 468A (0.200 g, 1.00 mmol) was suspended in acetic acid (5.0 mL) and iron powder (325 mesh, 0.112 g, 2.00 mmol) was added. The reaction was then heated at 70° C. for 20 min and then cooled to 22° C. The reaction was filtered through Celite, rinsing with ethyl acetate. The ethyl acetate rinse was collected and washed with sat. aq. K_2CO_3. The aqueous layer was extracted with ethyl acetate (3×20 mL) and the combined organic layers were dried over anhydrous magnesium sulfate. Concentration in vacuo gave 0.170 g (100%) of compound 468B as a yellow solid. HPLC: 88% at 1.677 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 171.29 $[M+H]^+$.

C. (3aα,4β,7β,7aα)-8-[Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-5-quinoxalinecarbonitrile (468C)

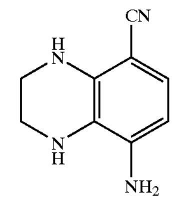
Compound 468B (0.060 g, 0.35 mmol) was suspended in toluene (1.0 mL) with magnesium sulfate (0.060 g) and compound 20A (0.104 g, 0.529 mmol). TEA (0.2 mL), was then added and the mixture was heated to 145° C. in a sealed tube. After 16 h the reaction was cooled to 22° C. and filtered through Celite, rinsing with acetone. The mixture was concentrated in vacuo and then purified by preparative TLC on silica gel eluting with 7% ethyl acetate/methylene chloride. This gave 0.018 g (15%) of compound 468C as a yellow solid. HPLC: 100% at 2.040 and 2.133 min (atropisomers, retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 349.33 $[M+H]^+$.

EXAMPLE 469

(3aα,4β,7β,7aα)-1,2,3,4-Tetrahydro-8-(octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-5-(quinoxalinecarbonitrile (469B)



A. 8-Amino-1,2,3,4-tetrahydro-quinoxaline-5-carbonitrile (469A)



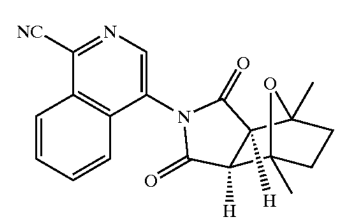
Compound 468A (0.037 g, 0.18 mmol) was dissolved in a mixture of ethyl acetate (1.0 mL)/ethanol (1.0 mL) and 10% Pd/C (0.050 g) was added. Hydrogen was then introduced via a balloon. After 2 h, the reaction was purged with nitrogen and filtered through Celite, rinsing with ethyl acetate. Concentration in vacuo gave 0.029 g (90%) of compound 469A as a red oil, which was taken on without further purification. HPLC: 97% at 3.217 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

B. (3aα,4β,7β,7aα)-1,2,3,4-Tetrahydro-8-(octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-5-quinoxalinecarbonitrile (469B)

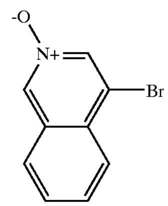
Compound 469A (0.029 g, 0.17 mmol) was suspended in toluene (1.0 mL) with magnesium sulfate (0.030 g) and compound 20A (0.050 g, 0.256 mmol). TEA (0.2 mL) was then added and the mixture was heated at 145° C. in a sealed tube. After 48 h the reaction was cooled to 22° C. and filtered through Celite, rinsing with acetone. The mixture was concentrated in vacuo and then purified by preparative TLC eluting with 20% acetone in chloroform. This gave 0.014 g (24%) of compound 469B as a yellow solid. HPLC: 85% at 2.267 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90%.aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 353.19 $[M+H]^+$.

EXAMPLE 470

(3aα,4β,7β,7aα)-4-(Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-1-isoquinolinecarbonitrile (470E)

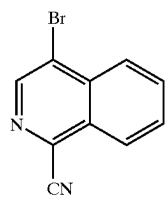


A. 4-Bromo-isoquinoline 2-oxide (470A)



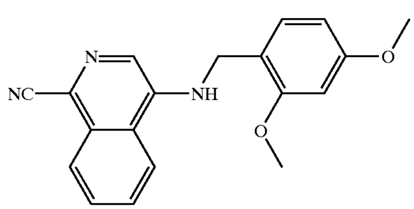
A solution of 4-bromoisoquinoline (4.16 g, 18.6 mmol) in 100 mL of chloroform was added dropwise over 1 h to a solution of 70% mCPBA (12.4 g, 50.3 mmol) in 100 mL of chloroform at room temperature. After stirring 18 h, the reaction mixture was washed with 1N NaOH (2×150 mL), dried over magnesium sulfate and concentrated in vacuo to afford 4.23 g (94%) of compound 470A as an off-white solid. ^{1}H NMR-400 MHz ($CDCl_3$): δ 8.71 (s, 1H), 8.43 (s, 1H), 8.09 (d, 1H, J=8 Hz), 7.70 (m, 3H).

B. 4-Bromo-isoquinoline-1-carbonitrile (470B)



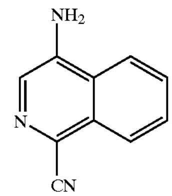
1,8-Diazabicyclo[5.4.0]undec-7-ene (1.67 mL, 11.2 mmol) was added to a mixture of compound 470A (1.12 g, 5.00 mmol) and cyanotrimethylsilane (0.75 mL, 5.5 mmol) in 35 mL of THF. The resulting homogeneous mixture was refluxed for 20 min. After concentrating in vacuo, the residue was purified by flash chromatography on a 5×15 cm silica get column, eluting with 3:1 hexane:ethyl acetate to give 0.95 g (82%) of compound 470B as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.85 (s, 1H), 8.36 (d, 1H, J=8.5 Hz), 8.28 (d, 1H, J=8.5 Hz), 7.96 (t, 1H, J=8.5 Hz), 7.89 (t, 1H, J=8.5 Hz).

C. 4-(2,4-Dimethoxy-benzylamino)-isoquinoline-1-carbonitrile (470C)



A mixture of compound 470B (699 mg, 3.00 mmol) and 2,4-dimethoxybenzylamine (4.8 mL, 30 mmol) in 15 mL of acetonitrile was refluxed for 16 h. After concentration in vacuo, the residue was purified on a 5×15 cm silica gel column, eluting with 3:2 hexane:ethyl acetate to afford 290 mg (30%) of 470C as a light yellow solid. HPLC: 1.76 min (retention time) (Phenomenex C-18, 5 micron column, 4.6×30 mm, eluting with 10–90% aqueous methanol over 2 min containing 0.1% TFA, 4 mL/min, monitoring at 254 nm).

D. 4-Amino-isoquinoline-1-carbonitrile (470D)



Compound 470C (50 mg, 0.16 mmol) was treated with trifluoroacetic acid (0.5 mL) for 1 h. The highly colored mixture was partitioned between ethyl acetate (30 mL) and 1N NaOH (30 mL). After washing with brine (15 mL), the organic layer was dried over magnesium sulfate and concentrated in vacuo to afford 24 mg (92%) of compound 470D as a yellow solid. HPLC: 99% at 1.09 min (retention time) (Phenomenex C-18, 5 micron column, 4.6×30 mm, eluting with 10–90% aqueous methanol over 2 min containing 0.1% TFA, 4 mL/min, monitoring at 254 nm). MS (ES^+): m/z 170.2 $[M+H]^+$.

An alternative route to the synthesis of compound 470D is as follows. A mixture of compound 470B (1.17 g, 5.02 mmol), benzophenone imine (1.05 mL, 6.26 mmol), palladium acetate (25 mg, 0.11 mmol), rac-2,2'-bis(diphenylphosphino)-1,1'binaphthyl (100 mg, 0.161 mmol) and cesium carbonate (2.30 g, 7.06 mmol) in 20 mL of toluene was heated at 100° C. for 20 h. The reaction mixture was diluted with ethyl ether (200 mL) and filtered through Celite. After concentrating the filtrate, the residue was dissolved in 120 mL of THF and treated with 40 mL of 1N HCl. After standing for 2 h at room temperature, the mixture was partitioned between ethyl acetate (150 mL) and 0.25 N NaOH (160 mL). After washing with brine (100 mL), the organic layer was dried over magnesium sulfate. The organic layer was filtered and ~50 g of celite was added to the filtrate. After concentration in vacuo, the powdery residue was purified by flash chromatography on a 5×15 cm silica gel column eluting with 1 L each of 1:1 ethyl acetate:hexane, 6:4 ethyl acetate:hexane and 8:2 ethyl acetate:hexane to give 450 mg (53%) of 470D as a yellow powder.

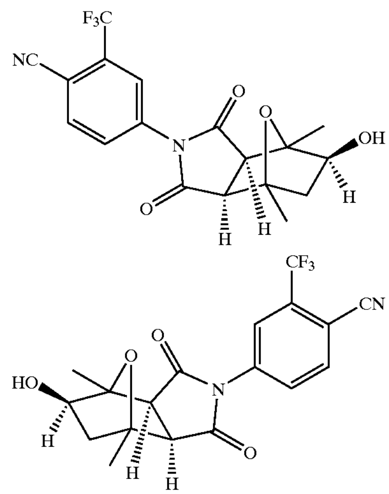
E. (3aα,4β,7β,7aα)-4-(Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-1-isoquinolinecarbonitrile (470E)

A mixture of compound 470D (24 mg, 0.14 mmol), compound 20A (55 mg, 0.28 mmol), triethylamine (0.1 mL), magnesium sulfate (100 mg); 2-methoxyethylether (0.5 mL) and DMF (0.1 mL) was heated in a sealed vessel to 250° C. for a total of 2.5 h using a microwave heating device. After partitioning the reaction mixture between ethyl acetate (25 mL) and water (25 mL), the organic layer was dried over magnesium sulfate and concentrated in vacuo. Approximately half of the residue was purified by reverse phase preparative HPLC (YMC S5 ODS 20×50 mm, eluting with 10–100% aqueous methanol over 10 min containing 0.1% TFA, 20 mL/min). Concentration of the pure fraction afforded 6 mg (12%) of compound 470E as a white powder. HPLC: 99% at 1.42 min (retention time) (Phenomenex

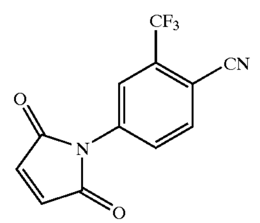
C-18, 5 micron column, 4.6×30 mm, eluting with 10–90% aqueous methanol over 2 min containing 0.1% TFA, 4 mL/min, monitoring at 254 nm). MS (ES+): m/z 348.23 $[M]^+$.

EXAMPLE 471

[3aR-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (471Di) & [3aS-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (471 Dii)

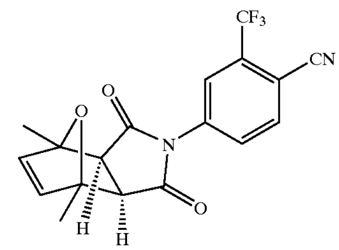


A. 4-(2,5-Dioxo-2,5-dihydro-pyrrol-1-yl)-2-trifluoromethyl-benzonitrile (471 A)



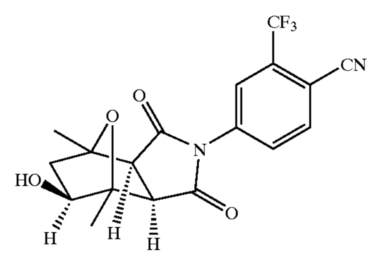
A mixture of 3-trifluoromethyl-4-cyano-aniline (24.0 g, 129 mmol) and maleic anhydride (14.0 g, 143 mmol) in 50 mL of acetic acid was heated at 115° C. overnight. A precipitate was obtained during the heating period. The reaction was allowed to stand at rt for an additional overnight period. The solid was removed by filtration, the filter cake was washed with diethyl ether and dried to give 21 g (79 mmol, 61%) of compound 471A as an off white solid. HPLC: 100% at 2.11 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

B. (3aα,4β,7β,7aα)-4-(1,3,3a,4,7,7a-Hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (471B)



A suspension of compound 471A (13.0 g, 48.8 mmol) and 2,5-dimethylfuran (10.5 mL, 98.6 mmol) in 50 mL of toluene was heated at 60° C., under argon. A solution was obtained on initial heating and a precipitate was observed after approximately 1 h. Heating was continued overnight. After cooling to rt, the suspension was allowed to stand at 4° C. overnight. The resulting solid was filtered and the filter cake was washed with cold toluene followed by air drying to give 13.2 g of pure compound 471B as a white solid. The filtrate volume was reduced in vacuo by one half and the resulting solution was treated as above to yield an additional 2.8 g of pure compound 471B (total 16.0 g, 90%). HPLC: 90% at 3.65 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

C. (3aα,4β,5β,7β,7aα)-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (471C)



A solution of compound 471B (25 g, 69 mmol) in 125 mL of THF, in a dry flask under nitrogen, was cooled to 10° C. with an ice bath. To this solution was added neat borane-dimethylsulfide complex (13.0 mL, 138 mmol) dropwise over 10 min, while maintaining a reaction temperature of <15° C. The reaction mixture was stirred for 30 min at rt and then in an ice bath cooled to 10° C. To the cool solution was slowly added 480 mL of pH 7 phosphate buffer, which resulted in a strong exothermic reaction and vigorous gas evolution. The solution was maintained at <21° C. throughout the addition by means of an ice bath. To the resulting solution was added 240 mL of ethanol and the resulting mixture was cooled to 5° C. with an ice bath. To the cooled solution was added 50 mL of 30% hydrogen peroxide and the resulting mixture was stirred at 10–20° C. for 1.5 h. The mixture was extracted with ethyl acetate (2×1 L) and the combined organic layers were washed with 10% sodium sulfite (1×500 mL) and brine (2×300 mL) and dried over $MgSO_4$. Concentration in vacuo afforded 29 g of crude product as a white solid. This material was subjected to flash

chromatography on a 1.2 L column of silica gel equilibrated with 100% CH_2Cl_2. The material was applied to the column as a solution consisting of 100 mL EtOAc (warm) and 400 mL CH_2Cl_2. Initial elution with CH_2Cl_2 (3 L), followed by 25% EtOAc/75% CH_2Cl_2 (3 L) and finally 50% EtOAc/50% CH_2Cl_2 (6 L) gave 11.8 g (45%) of compound 471C which is a racemic mixture.

Alternatively compound 471C can be made by the following approach: A dry flask containing compound 471B (8.90 g, 24.6 mmol) and Wilkinson's catalyst (0.57 mg, 0.62 mmol) was degassed 4× with vacuum/argon. THF (40 mL) was added to the flask and the mixture was stirred until a clear brown solution was obtained. Catecholborane (49 mL, 49 mmol, 1 M in THF) was then added dropwise over 20 min and a slight exotherm was observed. Stirring was continued for 45 min followed by cooling of the reaction mixture with an ice bath. pH 7 phosphate buffer (175 mL) was slowly added, followed by the consecutive addition of ethanol (87 mL) and 30% hydrogen peroxide (18 mL). Stirring was continued with cooling and the reaction progress was monitored by HPLC for 4 h. The reaction was extracted with CH_2Cl_2 (3×250 mL). The combined extracts were washed with 1:1 1N NaOH:15% sodium sulfite (300 mL) and brine, dried over $MgSO_4$, and the solvent was removed in vacuo to afford 8.5 g of a tan solid. The crude product was subjected to flash chromatography on a 500 cm^3 silica gel column eluting with a gradient of 25–50% EtOAc/CH_2Cl_2 to give 6.00 g compound 471C (15.8 mmol, 64%) as a white solid. HPLC: 90% at 2.45 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 381.11 $[M+H]^+$.

D. [3aR-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (471Di) & [3aS-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (471 Dii)

The individual antipodes of compound 471C were separated by normal phase preparative chiral HPLC (CHIRALPAK AD, 5×50 cm column). A 2.5 g portion of 471C was dissolved into 25 mL of warm acetone and diluted to 50–75 mL with hexane for injection. Isocratic elution with 20% MeOH/EtOH (1:1) in heptane at 50 mL/min gave the faster eluting compound 471Di (Chiral HPLC: 10.02 min; CHIRALPAK AD 4.6×250 mm column; isocratic elution with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min) and the slower eluting compound 471Dii (Chiral HPLC: 14.74 min; CHIRALPAK AD 4.6×250 mm column; isocratic elution with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min). Compounds 471Di & 471Dii: HPLC: 90% at 2.45 min (retention time) (YMC S5 ODS column, 4.6×50 min, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 381.11 $[M+H]^+$. The absolute stereochemistry of compounds 471Di & 471Dii was determined by single crystal X-ray diffraction studies and is as described by the designated nomenclature.

The resulting HPLC purified fractions of compounds 471Di & 471Dii were further purified by crystallization using any one of the procedures described below.

1) From Ethyl Acetate

A 700 mg portion of compound 471Di, obtained after chiral chromatography as described above, was dissolved in ethyl acetate (10 mL) at rt. The solution was diluted with small portions of hexane (20 mL) until cloudiness was observed. The solution was allowed to stand overnight at rt. The resulting white solid was filtered and air dried to afford 430 mg of compound 471Di as a white powder. This sample was further dried at 60° C. (3 h, 0.5 Torr), then at 70° C., (12 h, 0.5 Torr).

2) From Acetone

A 500 mg portion of compound 471Di, obtained after chiral chromatography as described above, was dissolved in a minimal amount of acetone (3 mL) and slowly diluted with hexane (1 mL). The clear colorless solution was allowed to stand overnight at rt. The resulting white solid was filtered and air dried to afford 440 mg of compound 471Di as a white powder. This sample was further dried at 60° C., (3 h, 0.5 Torr) then at 70° C., (12 h, 0.5 Torr).

3) From Methanol

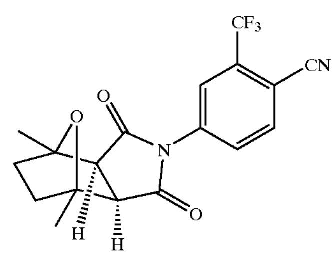
A 500 mg portion of compound 471Di, obtained after chiral chromatography as described above, was dissolved in 5 mL of hot (steam bath) methanol. The clear colorless solution was allowed to stand at rt for 2 h, then at 4° C. overnight. The resulting solid was filtered, washed with minimal cold methanol and air dried for to afford 360 mg of compound 471Di as a white powder. This sample was further dried at 70° C., (12 h, 0.5 Torr).

4) From CH_2Cl_2

A 7.00 g portion of compound 471Di, obtained after chiral chromatography as described above, was dissolved in 75 mL of CH_2Cl_2 at rt. The clear and colorless solution was slowly diluted with hexane (48 mL) until crystallization was observed. The solution was allowed to stand at rt for 1 h, then at 4° C. overnight. The resulting crystalline material was filtered and then washed with a minimal amount of cold 2:1 CH_2Cl_2:hexane. The large crystals were ground to a fine powder and dried at 50° C. (12 h, 0.5 Torr) to yield 5.96 g of compound 471Di as a white powder.

EXAMPLE 472

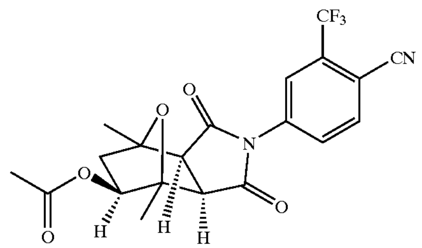
(3aα,4β,7β,7aα)-4-(Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (472)



A solution of compound 471B (500 mg, 1.38 mmol) in ethyl acetate (10 mL), containing 10% Pd/C (25 mg, cat.) was stirred at rt under an atmosphere of hydrogen introduced via a balloon. After 2 h the reaction was filtered through Celite and the filter cake was washed with EtOAc. The clear, colorless filtrate was concentrated in vacuo to yield 501 mg (1.38 mmol, 100%) of compound 472 as a white solid. No further purification was required. HPLC: 99% at 3.04 min (retention time) (YMC S5 ODS column, 4.6×50 mm, 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ESI): m/z 382.2 $[M+NH_4]^+$.

EXAMPLE 473

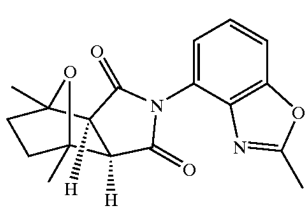
(3aα,4β,5β,7β,7aα)-4-[5-(Acetyloxy)octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (473)



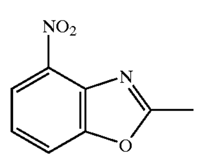
To a solution of compound 471C (1.50 g, 3.95 mmol) in 10 mL of pyridine, cooled to 0° C. under argon, was added acetic anhydride (0.42 mL, 4.4 mmol) dropwise, followed by DMAP (5 mg, 0.04 mmol). Stirring was continued at rt for 4 h. The solution was concentrated in vacuo and the resulting residue was diluted with ethyl acetate, and washed consecutively with 1N HCl (2×), brine (2×), sat. $NaHCO_3$, and brine (2×). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The resulting solid was dried at 60° C. (20 h, 0.5 Torr) to yield 1.55 g (3.67 mmol, 93%) of compound 473 as a white crystalline solid. HPLC: 99% at 2.10 min (retention time) (Phenomenex Luna C18 column, 2×30 mm, 0–100% aqueous acetonitrile over 3 min containing 10 mM NH_4OAc at 1 mL/min, monitoring at 220 nm). MS (ESI): m/z 421.4 $[M-H]^-$.

EXAMPLE 474

(3aβ,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-(2-methyl-4-benzoxazolyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione (474F)

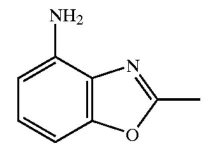


A. 2-Methyl-4-nitrobenzoxazole (474A)



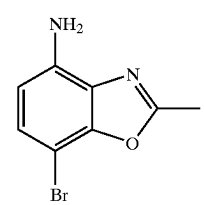
To 2-amino-3-nitrophenol (6.17 g, 40.0 mmol) was added triethylorthoacetate (25.96 g, 160.0 mmol) and the mixture was heated at 100° C. for 12 h to give a dark red solution. Cooling to room temperature produced a crystalline mass which was filtered and washed with hexane to give compound 474A (6.78 g, 95%) as light maroon needles. HPLC: 98.1% at 1.86 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 179.08 $[M+H]^+$.

B. 4-Amino-2-methylbenzoxazole (474B)



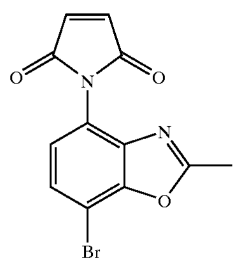
Compound 474A (6.78 g, 38.1 mmol) was dissolved in a 1:1 mixture of 10% acetic acid/ethyl acetate (100 mL total volume) and heated to 65° C. Iron powder (10.63 g, 190.2 mmol) was added portionwise. After stirring for 3 h, TLC indicated complete consumption of starting material. The cooled reaction mixture was filtered through a pad of Celite and the pad was washed with 50 mL of ethyl acetate. The organic layer was separated, washed with water (2×25 mL), brine (1×25 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The crude material was purified by flash chromatography on silica gel eluting with 25% ether/CH_2Cl_2 to give 3.90 g (69%) of compound 474B as a light brown solid. HPLC: 95.8% at 2.43 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 149.11 $[M+H]^+$.

C. 4-Amino-7-bromo-2-methylbenzoxazole (474C)



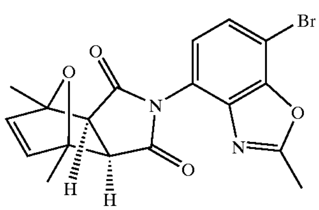
Compound 474B (3.90 g, 26.3 mmol) was dissolved in DMF (45 mL) and cooled to −5° C. and N-bromosuccinimide (4.68 g, 26.3 mmol) was added in small portions and the reaction stirred for 5 h. The mixture was poured into 150 mL of ice water to give a cream colored solid which was filtered, washed with water, dissolved in CH_2Cl_2, dried over $MgSO_4$, filtered and concentrated in vacuo. Purification of the crude material by flash chromatography on silica gel eluting with 20% ether/CH_2Cl_2 gave compound 474C (3.36 g, 56%) as a beige solid. HPLC: 95.4% at 2.583 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 228.03 $[M+H]^+$.

D. 1-(7-Bromo-2-methyl-benzoxazol-4-yl)-pyrrole-2,5-dione (474D)



Compound 474C (1.40 g, 6.17 mmol) was dissolved in 20 mL of acetic acid, maleic anhydride (0.635 g, 6.47 mmol) was added and the reaction was heated at reflux under nitrogen for 5 h. The solvent was removed in vacuo and the crude product was purified by flash chromatography on silica gel eluting with 10% ether/CH_2Cl_2 to give compound 474D (1.73 g, 91%) as a pale yellow solid. HPLC: 93.6% at 1.36 min. (Phenomenex column, 30×4.6 mm, 10–90% aqueous methanol over 2 min containing 0.1% TFA, 5 mL/min, monitoring at 220 nm. MS (ES): m/z 308.02 $[M+H]^+$.

E. (3aα,4β,7β,7aα)-2-(7-Bromo-2-methyl-4-benzoxazolyl)-3a,4,7,7a-tetrahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (474E)



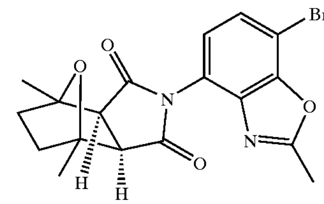
Compound 474D (0.307 g, 1.00 mmol) was dissolved in benzene (2 mL) and 2,5-dimethylfuran (0.154 g, 1.60 mmol) was added via syringe. The reaction mixture was heated to 60° C. for 12 h. The cooled reaction mixture was concentrated in vacuo at 40° C. to give compound 474E as an off-white foam which was used directly in the next reaction without purification.

F. (3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-(2-methyl-4-benzoxazolyl)-4,7-epoxy-1H-Isoindole-1,3 (2H)-dione (474F)

Compound 474E (0.403 g, 1.00 mmol) was dissolved in EtOH/EtOAc (4 mL/4 mL) and 10% Pd/C (100 mg) was added. The reaction mixture was stirred at room temperature for 6 h under an atmosphere of H_2 supplied by a balloon and then filtered through Celite. Concentration of the filtrate in vacuo gave a brown solid. Purification by flash chromatography on silica gel eluting with 10% acetone/$CHCl_3$ (250 mL), 15% acetone/$CHCl_3$ (250 mL), and 20% acetone/$CHCl_3$ (250 mL) gave compound 474F (0.089 g, 27%) as a white foam. HPLC: 91% at 2.28 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 328.34 $[M+H]^+$.

EXAMPLE 475

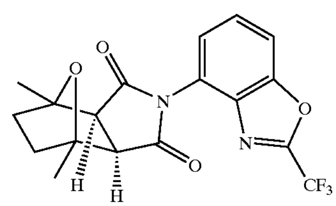
(3aα,4β,7β,7aα)-2-(7-Bromo-2-methyl-4-benzoxazolyl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (475)



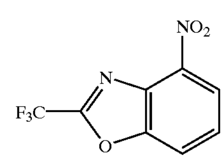
Compound 474E (0.202 g, 0.501 mmol) was dissolved in 1/1 EtOAc/EtOH (10 mL) and 10% Pt/C (100 mg) was added. The reaction mixture was stirred at room temperature under an H_2 balloon for 6 h. The reaction was filtered through Celite and concentrated in vacuo. Purification by flash chromatography on silica gel eluting with 10% ether/CH_2Cl_2 gave 0.063 g (31%) of compound 475 as a colorless oil which solidified upon standing to give a white solid. HPLC: 92.5% at 2.83 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 406.21 $[M+H]^+$.

EXAMPLE 476

(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-[2-(trifluoromethyl)-4-benzoxazolyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione (476D)

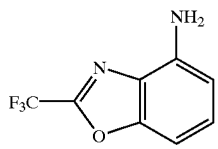


A. 4-Nitro-2-trifluoromethylbenzoxazole (476A)



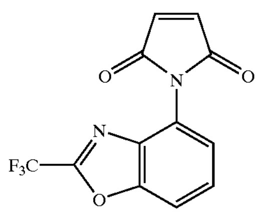
2-Amino-3-nitrophenol (10.00 g, 64.88 mmol) was added to 100 mL of vigorously stirring trifluoroacetic anhydride and the resulting mixture was stirred at room temperature for 12 h. The solvent was removed in vacuo to give a dark blue solid which was dissolved in 200 mL of CH_2Cl_2 and washed sequentially with 10% NaOH (2×100 mL), water (100 mL), brine (100 mL), and dried over $MgSO_4$. Filtration and concentration in vacuo gave compound 476A (10.78 g, 72%) as a brown solid. No further purification was required. HPLC: 92.9% at 2.43 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

B. 4-Amino-2-trifluoromethylbenzoxazole (476B)



Compound 476A (10.75 g, 46.30 mmol) was dissolved in 1:1 EtOAc/10% HOAc (250 mL) and heated to 65° C. Iron powder (12.93 g, 231.5 mmol) was added portionwise and the reaction was stirred for 6 h at 65° C. After cooling, the mixture was filtered through Celite rinsing with EtOAc. The organic layer was separated, washed with H_2O (3×100 mL), brine (100 mL), dried over $MgSO_4$, and concentrated in vacuo to give a brown oil. The crude material was purified by flash chromatography on silica gel eluting with 70/30 CH_2Cl_2/hexanes to give compound 476B (7.02 g, 75%) as a yellow crystalline solid. HPLC: 96.7% at 2.68 nin (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 nin containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

C. 1-(2-Trifluoromethyl-benzoxazol-4-yl)-pyrrole-2,5-dione (476C)



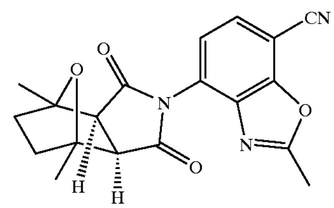
Compound 476B (0.500 g, 2.48 mmol) was dissolved in acetic acid (10 mL) and maleic anhydride (0.267 g, 2.72 mmol) was added. The mixture was heated at reflux for 3 h, cooled and the solvent removed in vacuo to give a tan solid. The crude product was purified by flash chromatography on silica gel eluting with 2% $MeOH/CH_2Cl_2$ to give compound 476C (0.40 g, 57%) as an off-white solid. HPLC: 89.7% at 2.38 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 283.21 $[M+H]^+$.

D. (3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-[2-(trifluoromethyl)-4-benzoxazolyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione (476D)

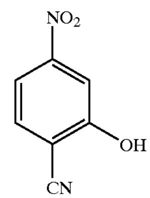
Compound 476C (0.24 g, 0.85 mmol) and 2,5-dimethylfuran (0.132 g, 1.37 mmol) were combined in 3 mL of benzene in a sealed tube and heated at 60° C. for 12 h. The mixture was cooled and concentrated in vacuo to give a yellow oil which was dissolved in 1/1 EtOAc/EtOH (6 mL). 10% Pd/C (100 mg) was added and the mixture was stirred under an H_2 balloon for 3.5 h. The reaction was filtered through Celite and the solvent removed in vacuo to give the crude product as a pale yellow oil. Purification by flash chromatography on silica gel eluting with 2% Et_2O/CH_2Cl_2 gave 0.107 g (33%) of compound 476D as a white foam. HPLC: 96.5% at 2.80 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 ml/min, monitoring at 220 nm). MS (ES): m/z 381.17 $[M+H]^+$.

EXAMPLE 477

(3aα,4β,7β7aα)-2-Methyl-4-(octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-7-benzoxazolecarbonitrile (477E)

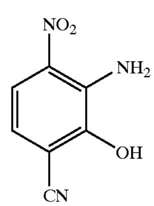


A. 2-Cyano-5-nitrophenol (477A)



3,4-Methylenedioxynitrobenzene (1.67 g, 10.0 mmol) was dissolved in 20 mL of HMPA and sodium cyanide (0.49 g, 10.0 mmol) was added. The reaction was heated to 150° C. under nitrogen and three portions of sodium cyanide (0.245 g, 5.00 mmol, total) were added over 15 min. The reaction was maintained at 150° C. for 45 min, cooled, and poured into 50 mL of H_2O followed by the addition of 50 mL of 5% NaOH. The aqueous layer was extracted with ether (2×25 mL) and the organic layer was discarded. The basic aqueous layer was carefully acidified to pH 4 by addition of 10% HCl and extracted with ether (3×25 mL). The combined organic layers were washed with brine (25 mL), dried over sodium sulfate and concentrated in vacuo. Purification by flash chromatography on silica gel eluting with 5% $MeOH/CH_2Cl_2$ gave 1.05 g (64%) of compound 477A as a yellow-brown solid. HPLC: 91.6% at 1.03 min (retention time) (Phenomenex column, 30×4.6 mm, 10–90% aqueous methanol over 2 min containing 0.1% TFA, 5 mL/min, monitoring at 220 nm. MS (ES): m/z 165.23 $[M+H]^+$.

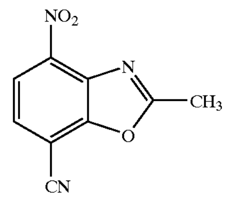
B. 2-Amino-4-cyano-3-hydroxynitrobenzene (477B)



Compound 477A (0.438 g, 2.67 mmol) was dissolved in 25 mL of DMSO and trimethylhydrazinium iodide (0.534 g, 2.67 mmol) was added. Sodium pentoxide (0.880 g, 8.01 mmol) was added under N_2 to give a deep red solution and stirring was continued overnight at rt. The reaction mixture was poured into 50 mL of 10% HCl and extracted with EtOAc (2×25 mL). The combined organic layers were washed with water (25 mL), brine (25 mL), dried over sodium sulfate and concentrated in vacuo to give compound

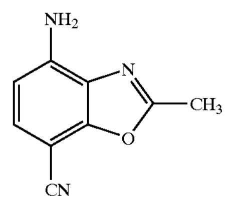
477B as an oily red solid which was used directly in the next reaction without further purification.

C. 7-Cyano-2-methyl-4-nitrobenzoxazole (477C)



Compound 477B (0.360 g, 2.01 mmol) and triethyl orthoacetate (1.30 g, 8.04 mmol) were combined and heated at reflux under nitrogen for 1 h. The solvent was removed in vacuo and the resulting residue purified by flash chromatography eluting with 5% ether/CH_2Cl_2 to give 0.255 g (63%) of compound 477C as a brown solid. HPLC: 98.4% at 1.80 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 204.28 $[M+H]^+$.

D. 4-Amino-7-cyano-2-methylbenzoxazole (477D)



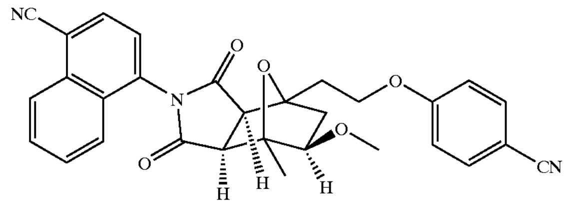
Compound 477C (0.156 g, 0.77 mmol) was dissolved in a 1:1 mixture of EtOAc/10% HOAc (20 mL) and heated to 65° C. Iron powder (325 mesh, 0.214 g, 3.83 mmol) was added and the reaction was stirred for 4 h. The cooled mixture was filtered through Celite and the resulting filtrate was washed with water (25 mL), brine (25 mL), dried over $MgSO_4$, and concentrated to give compound 477D (0.118 g, 89%) as an orange solid. HPLC: 87% at 2.03 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 174.05 $[M+H]^+$.

E. (3aα,4β,7β,7aα)-2-Methyl-4-(octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-7-benzoxazolecarbonitrile (477E)

Compound 477D (0.060 g, 0.35 mmol) and compound 20A (0.071 g, 0.37 mmol) were combined in a sealed tube with toluene (2 mL), triethylamine (0.24 mL, 1.7 mmol), and $MgSO_4$ (0.104 g, 0.866 mmol). The sealed tube was heated at 135° C. for two days. The cooled reaction mixture was diluted with EtOAc, filtered, and concentrated in vacuo to give crude product as a brown oil. Purification by flash chromatography on silica gel eluting with 1/1 EtOAc/hexanes gave 0.014 g (12%) of compound 477E as an off-white solid. HPLC: 96.5% at 2.27 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 352.23 $[M+H]^+$.

EXAMPLE 478

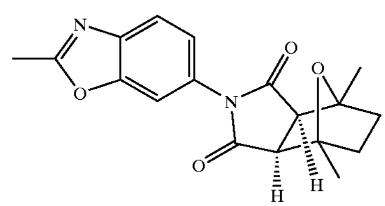
(3aα,4β,5β,7β,7aα)-4-[7-[2-(4-Cyanophenoxy)ethyl]octahydro-5-methoxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, Slow Eluting Enantiomer (478)



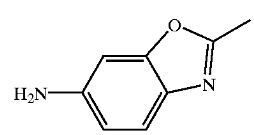
n-BuLi (0.050 mL, 1.6 M, 0.075 mmol) was added to a solution of compound 244ii (33.7 mg, 0.0683 mmol) in THF (1.0 mL) at −78° C. under argon. The reaction mixture was warmed to room temperature and methyl fluorosulfonate (0.010 mL, 0.12 mmol) was added dropwise. Once starting material was consumed, as was evident by HPLC, the reaction was quenched with H_2O and the resulting aqueous mixture was extracted with CH_2Cl_2 (3×5 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by reverse phase preparative HPLC [22.09 min (YMC S5 ODS column, 20×100 mm, 0–100% aqueous methanol over 25 min containing 0.1% TFA, 20 mL/min, monitoring at 220 nm)] gave 13.0 mg (38%) of compound 478 as a white solid. HPLC: 93% at 3.35 min (YMC S5 ODS column, 4.6×50 mm, 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 508.17 $[M+H]^+$.

EXAMPLE 479

(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-(2-methyl-6-benzoxazolyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione (479B)



A. 2-Methyl-6-aminobenzoxazole (479A)



To a solution of 2-methyl-6-nitrobenzoxazole (100 mg, 0.560 mmol) in AcOH (2 mL) was added iron powder (325 mesh, 63.0 mg, 1.12 mmol) at 70° C. in a single portion. After 15 min at 70° C. additional iron powder (325 mesh, 63.0 mg, 1.12 mmol) was added and stirring was continued for 15 min. The mixture was cooled and concentrated under reduced pressure. The resulting residue was taken up into EtOAc and washed with sat. Na_2CO_3 followed by H_2O. The

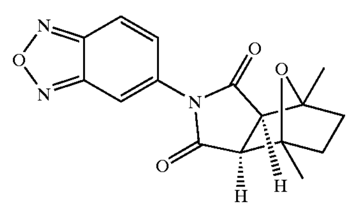
organic layer was dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography on silica gel eluting with a gradient of 0 to 25% EtOAc in CH_2Cl_2 to yield 69 mg (83%) of compound 479A as a solid. HPLC: 97% at 0.24 min (retention time) (YMC S5 ODS column, 4.6×50 mm Ballistic, 10–90% aqueous methanol over 4 min containing 0.2% H_3PO_4, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 149.2 $[M+H]^+$.

B. (3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-(2-methyl-6-benzoxazolyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione (479B)

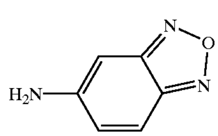
Compound 479A (30 mg, 0.20 mmol), $MgSO_4$ (60 mg, 0.50 mmol), triethylamine (140 μL, 1.00 mmol) and compound 20A (45 mg, 0.23 mmol) were taken up in 0.25 mL of toluene and placed in a sealed tube. The sealed tube was heated at 135° C. for 14 h and the reaction was allowed to cool to rt. The mixture was filtered through a short pad of Celite, eluting with MeOH and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel eluting with a gradient of 0 to 50% EtOAc in CH_2Cl_2 to give 49 mg (65%) of compound 479B as a tan solid. HPLC: 98% at 2.30 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/Z 326.9 $[M+H]^+$.

EXAMPLE 480

(3aα,4β,7β,7aα)-2-(2,1,3-Benzoxadiazol-5-yl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (480B)



A. 5-Amino-2,1,3-benzoxadiazole (480A)



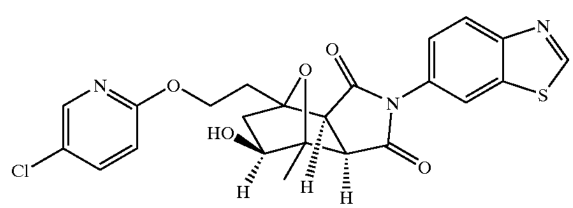
To a solution of 2,1,3-benzoxadiazole-5-carboxylic acid (102 mg, 0.621 mmol) in THF (3 mL) was added triethylamine (103 μL, 0.739 mmol) followed by DPPA (160 μL, 0.739 mmol) at room temperature. The mixture was stirred for 4 h, diluted with CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, concentrated and purified by flash chromatography on silica gel with 0 to 50% EtOAc in CH_2Cl_2. The resulting material was dissolved in AcOH (2 mL) and water (0.7 mL) was added dropwise yielding a slightly cloudy solution which was heated at 105° C. for 30 min. The mixture was cooled, made basic with sat. Na_2CO_3 solution and extracted several times with THF. The combined organic layers were dried over $MgSO_4$, concentrated and purified by flash chromatography on silica gel eluting with 0 to 15% MeOH in CH_2Cl_2 to give 34 mg (41%) of compound 480A as a yellow solid. HPLC: 100% at 1.27 min (retention time) (YMC S5 ODS column, 4.6×50 mm Ballistic, 10–90% aqueous methanol over 4 min containing 0.2% H_3PO_4, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 136.0 $[M+H]^+$.

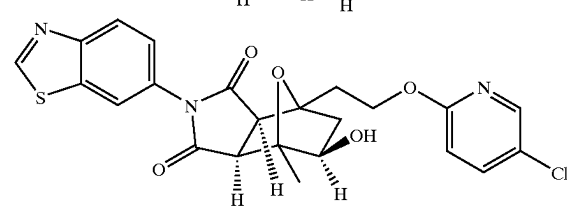
B. (3aα,4β,7β,7aα)-2-(2,1,3-Benzoxadiazol-5-yl)hexahydro-4,7-dimethyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (480B)

Compound 480A (34 mg, 0.25 mmol), $MgSO_4$ (76 mg, 0.63 mmol), triethylamine (180 μL, 1.26 mmol) and compound 20A (74 mg, 0.38 mmol) were dissolved in 0.25 mL of toluene and placed in a sealed tube. The sealed tube was heated at 135° C. for 14 h. The cooled reaction mixture was filtered through a short pad of Celite, eluting with acetone and the solvent was removed in vacuo. The residue was purified by reverse phase preparative HPLC (YMC S5 ODS 20×100 mm, eluting with 30–100% aqueous methanol over 10 min containing 0.1% TFA, 20 mL/min). Concentration of the desired fractions afforded a residue which was partitioned between CH_2Cl_2 and sat. $NaHCO_3$ solution. The aqueous layer was extracted once with CH_2Cl_2 and the combined organic phases were dried over Na_2SO_4. Concentration under reduced pressure gave 42 mg (53%) of compound 480B as a yellow solid. HPLC: 100% at 2.62 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). ^1H NMR (400 MHz, $CDCl_3$) δ=7.91 (d, 1H), 7.90 (dd, 1H), 7.37 (dd, 1H), 3.09 (s, 2H), 1.85 (s, 4H), 1.67 (s, 6H).

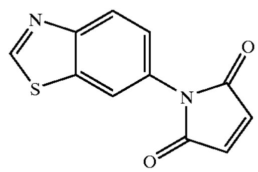
EXAMPLE 481

[3aR-(3aβ,4β,5β,7β,7aα)]-2-(6-Benzothiazolyl)-7-[2-[(5-chloro-2-pyridinyl)oxy]ethyl]hexahydro-5-hydroxy-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (481D) & [3aS-(3aα,4β,5β,7β,7aα)]-2-(6-Benzothiazolyl)-7-[2-[(5-chloro-2-pyridinyl)oxy]ethyl]hexahydro-5-hydroxy-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (481E)



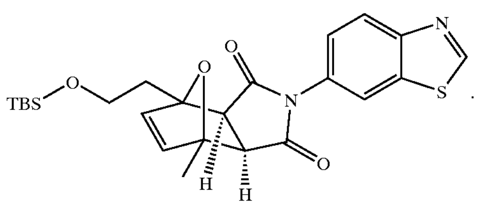


A. 1-Benzothiazol-6-yl-pyrrole-2,5-dione (481A)



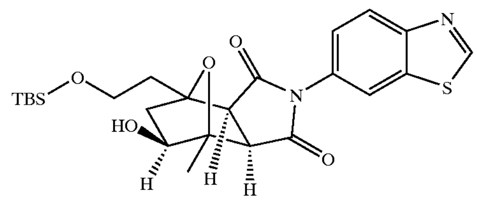
A mixture of 5-aminobenzothiazole (2.00 g, 13.3 mmol) and maleic anhydride (1.96 g, 20.0 mmol) in AcOH (27 mL) was heated at 115° C. for 20 h. The mixture was cooled and concentrated under reduced pressure. The residue was taken up in THF and washed with saturated Na_2CO_3. The aqueous layer was extracted several times with THF and the combined organic layers were dried over $MgSO_4$. Purification by flash chromatography on silica gel eluting with 0 to 50% EtOAc in CH_2Cl_2 gave 1.37 g (45%) of compound 481A as a pale yellow solid. HPLC: 100% at 2.62 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 231.0 $[M+H]^+$.

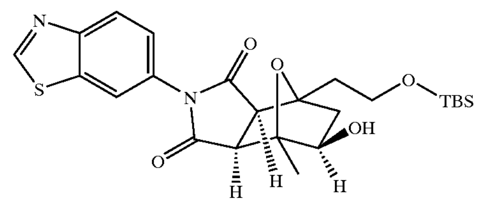
B. (3aα,4β,7β,7aα)-2-(6-Benzothiazolyl)-4-[2-[[(1,1-dimethylethyl)dimethylsilyl]oxy]ethyl]-3a,4,7,7a-tetrahydro-7-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (481B)



A suspension of compound 481A (445 mg, 1.93 mmol) and compound 204A (929 mg, 3.87 mmol) in benzene (2 mL) was heated to 60° C. and acetone was added until a clear solution was obtained. The resulting mixture was stirred at 60° C. for 24 h and was then slowly concentrated in vacuo. The resulting residue was dissolved in acetone and slowly concentrated in vacuo. This process was repeated a total of three times. Purification by flash chromatography on silica gel eluting with 0 to 30% acetone in hexanes gave 820 mg (90%) of compound 481B as a white solid. HPLC: 100% at 2.62 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 471.3 $[M+H]^+$.

C. [3aR-(3aα,4β,5β,7β,7aα)]-2-(6-Benzothiazolyl)-7-[2-[[(1,1-dimethylethyl)dimethylsilyl]oxy]ethyl]hexahydro-5-hydroxy-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (481Ci) & [3aS-(3aα,4β,5β,7β,7aα)]-2-(6-Benzothiazolyl)-7-[2-[[(1,1-dimethylethyl)dimethylsilyl]oxy]ethyl]hexahydro-5-hydroxy-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (481Cii)





To a solution of compound 481B (75 mg, 0.16 mmol) in THF (1 mL) was added Wilkinson's catalyst (32 mg, 0.030 mmol) and catecholborane (1.0 M in THF, 1.6 mL, 1.6 mmol) at room temperature under nitrogen. The resulting mixture was stirred for 2.5 h before it was cooled to 0° C. EtOH (5 mL), 3 N NaOH (2 mL) and H_2O_2 (30%, 1 mL) were added sequentially, and the mixture was stirred for 2 h at 0° C. The reaction was quenched by the addition of cold 10% Na_2SO_3 solution (excess) followed by water. The aqueous layer was extracted several times with CH_2Cl_2 and the combined organic layerss were dried over Na_2SO_4. Concentration under reduced pressure followed by purification by flash chromatography on silica gel eluting with 0 to 100% EtOAc in hexanes gave 13 mg (17%) of a racemic mixture of compounds 481Ci & 481Cii as a tan solid. HPLC: 96% at 3.58 min (retention time) (YMC S5 ODS column, 4.6×50 mm Ballistic, 10–90% aqueous methanol over 4 min containing 0.2% H_3PO_4, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 489.3 $[M+H]^+$. The racemic mixture was separated into its individual enantiomers by normal phase preparative chiral HPLC (CHIRALPAK AD 5×50 cm column; eluting with 20% MeOH/EtOH (1:1) in heptane (isocratic) at 50 mL/min) to give the faster eluting enantiomer, compound 481Ci: (Chiral HPLC: 9.40 min; CHIRALPAK AD 4.6×250 mm column; eluting with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min) and the slower eluting enantiomer, compound 481Cii: (Chiral HPLC: 10.47 min; CHIRALPAK AD 4.6×250 mm column; eluting with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min). The absolute conformation for compounds 481Ci & 481Cii was not established. For simplicity in nomenclature, compound 481Ci is designated herein as having an "R" configuration and compound 481Cii as having an "S" configuration. Enantiomerically pure products derived from compound 481Ci are designated herein as having a "R" configuration and enantiomerically pure products derived from compound 481Cii are designated herein as having an "S" configuration.

D. [3aR-(3aα,4β,5β,7β,7aα)]-2-(6-Benzothiazolyl)-7-[2-[(5-chloro-2-pyridinyl)oxy]ethyl]hexahydro-5-hydroxy-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (481D)

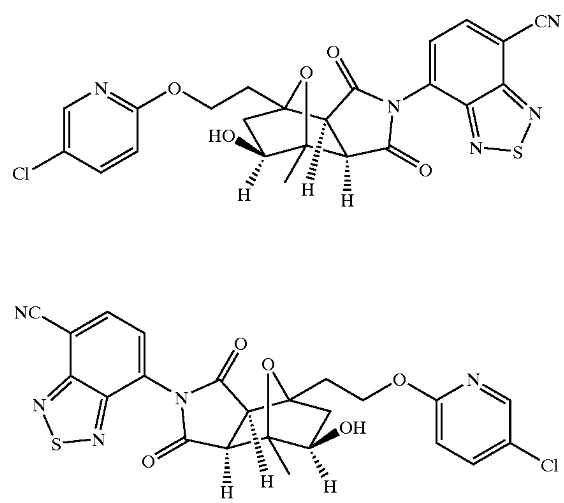
Compound 481Ci (84 mg, 0.17 mmol) was suspended into EtOH (2 mL) and conc. HCl (40 μL) was added at roomtemperature. The mixture was stirred for 15 min before several drops of sat. $NaHCO_3$ solution were added. Concentration under reduced pressure yielded a residue which was partitioned between CH_2Cl_2 and sat. $NaHCO_3$ solution. The aqueous layer was extracted several times with CH_2Cl_2 and finally with EtOAc. The combined organic phases were dried over Na_2SO_4, concentrated and purified by preparative TLC eluting with 50% acetone in $CHCl_3$. This procedure served to remove the TBS group from compound 481Ci, yielding the free primary alcohol. A 12 mg (0.03 mmol) portion of the free alcohol of compound 481Ci was reacted with 5-chloro-2-pyridinol (8 mg, 0.06 mmol), PPh_3 (17 mg, 0.060 mmol) and di-tert-butylazodicarboxylate (15 mg, 0.060 mmol) in THF (0.5 mL) according to the general procedure described in Example 244. The mixture was stirred for 24 h at room temperature, diluted with 1N NaOH and the aqueous layer was extracted several times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, concentrated and purified by preparative TLC, eluting with 25% acetone in $CHCl_3$ to give 9 mg (58%) compound 481D as a white solid. HPLC: 98% at 2.94 min (retention time) (YMC S5 ODS column, 4.6×50 mm Ballistic, 10–90% aqueous methanol over 4 min containing 0.2% H_3PO_4, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 486.2 $[M+H]^+$.

E. [3aS-(3aα,4β,5β,7β,7aα)]-2-(6-Benzothiazolyl)-7-[2-[(5-chloro-2-pyridinyl)oxy]ethyl]hexahydro-5-hydroxy-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (481E)

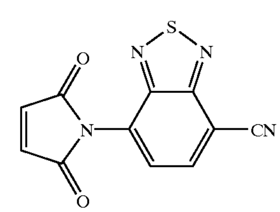
As described in Example 481D, compound 481Cii (58 mg, 0.12 mmol) was treated with EtOH (2 mL) containing 12 N HCl (40 μL) to yield the free primary alcohol product of compound 481Cii. A 15 mg (0.040 mmol) of the free alcohol of compound 481Cii was reacted with 5-chloro-2-pyridinol (10 mg, 0.080 mmol), PPh_3 (21 mg, 0.080 mmol) and di-tert-butylazodicarboxylate (18 mg, 0.080 mmol) in THF (0.5 mL) in the manner described above and the resulting product was purified as described above to yield 8 mg (41%) of compound 481E as a white solid. HPLC: 99% at 2.93 min (retention time) (YMC S5 ODS column, 4.6×50 mm Ballistic, 10–90% aqueous methanol over 4 min containing 0.2% H_3PO_4, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 486.2 $[M+H]^+$.

EXAMPLE 482

[3aR-(3aα,4β,5β,7β,7aα)]-7-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile (482F) & [3aS-(3aα,4β,5β,7β,7aα)]-7-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile (482G)

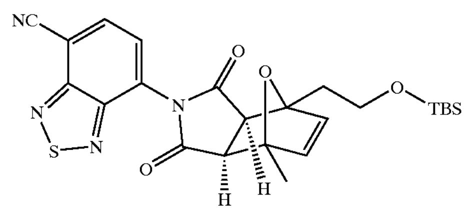


A. 7-(2,5-Dioxo-2,5-dihydro-pyrrol-1-yl)-benzo[1,2,5]thiadiazole-4-carbonitrile (482A)



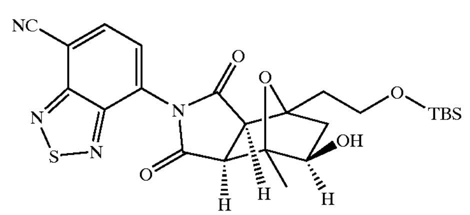
Maleic anhydride (667 mg, 6.80 mmol) was added to a solution of compound 424A (600 mg, 3.41 mmol) in THF (9 mL). The mixture was heated at 110° C. for 10 h. The reaction was concentrated under reduced pressure and acetic anhydride (1 ml) was added to the residue. The reaction mixture was heated at 75° C. for 30 min and then cooled to rt. Purification by flash chromatography on silica gel eluting with 3% acetone/$CHCl_3$ gave 758 mg (2.96 mmol, 67%) of compound 482A. HPLC: 97% at 1.98 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 min gradient with 0.2% H_3PO_4, monitoring at 220 nm). MS (ES): m/z 257.01 $[M+H]^+$.

B. (3aα,4β,7β,7aα)-7-[4-[2-[[(1,1-Dimethylethyl) dimethylsilyl]oxy]ethyl]-1,3,3a,4,7,7a-hexahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile (482B)



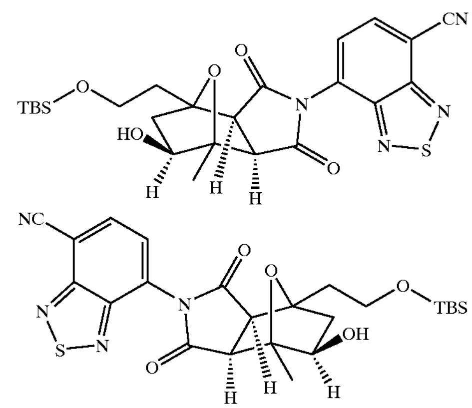
A solution of compound 482A (758 mg, 2.96 mmol) and compound 204A (711 mg, 2.96 mmol) in benzene (2 mL) and acetone (2 mL) was heated at 60° C. for 6 h. The reaction mixture was concentrated in vacuo at 42° C. for 40 min to give 1.5 g of crude compound 482B, which was used directly in the next step without further purification.

C. (3aα,4β,5β,7β,7aα)-7-[7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile (482C)



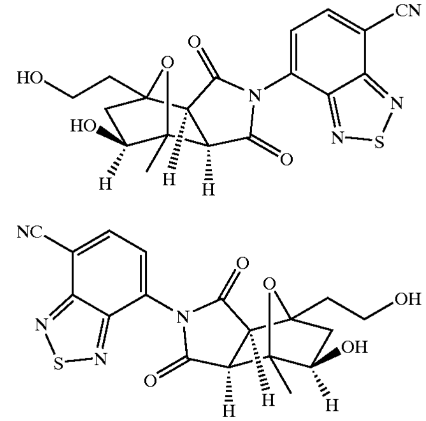
Borane-dimethylsulfide complex (0.66 mL, 6.96 mmol) was added to a solution of compound 482B (1.15 g, 2.32 mmol) in THF (6 mL) at 0° C. After stirring at 0° C. for 2 h, the reaction mixture was quenched with phosphate buffer (60 mL, pH 7.2) and then EtOH (35 mL), H_2O_2 (8 mL, 30% aq.) and THF (4 mL) were added. The reaction mixture was stirred at 0° C. for 1 h and was then extracted with CH_2Cl_2 (4×100 mL). The combined organic layers were washed with 10% aq. Na_2SO_3 (1×160 mL) followed by brine (1×160 mL) and dried over Na_2SO_4. Purification by flash chromatography on silica gel eluting with 10% acetone/$CHCl_3$ gave 250 mg (0.486 mmol, 21%) of compound 482C as an orange solid. HPLC: 85% at 3.70 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 min gradient with 0.2% H_3PO_4, monitoring at 220 nm). MS (ES): m/z 515.27 $[M+H]^+$.

D. [3aR-(3aα,4β,5β,7β,7aα)]-7-[7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile & [3aS-(3aα,4β,5β,7β,7aα)]-7-[7-[2-[[(1,1-Dimethylethyl) dimethylsilyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile (482Di & 482Dii)



The racemic compounds 482C was separated by normal phase preparative chiral HPLC using a Chiracel OD column (5 cm×50 cm), eluting with 10% EtOH in hexane at 50 mL/min to give the faster eluting compound 482Di (Chiral HPLC: 11.89 min; CHIRALCEL OD 4.6×250 mm column; isocratic elution with 12% EtOH in hexane at 2 mL/min) and the slower eluting compound 482Dii (Chiral HPLC-16.10 min; CHIRALCEL OD 4.6×250 mm column; isocratic elution with 12% EtOH in hexane at 2 mL/min). The absolute conformation for compounds 482Di & 482Dii was not established. For simplicity in nomenclature, compound 482Di is designated herein as having an "R" configuration and compound 482Dii as having an "S" configuration. Enantiomerically pure products derived from compound 482Di are designated herein as having a "R" configuration and enantiomerically pure products derived from compound 482Dii are designated herein as having an "S" configuration.

E. [3aR-(3aα,4β,5β,7β,7aα)]-7-[Octahydro-5-hydroxy-7-(2-hydroxyethyl)-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile (482Ei) & [3aR-(3aα,4β,5β,7β,7aα)]-7-[Octahydro-5-hydroxy-7-(2-hydroxyethyl)-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile (482Eii)



Compound 482Di (91 mg, 0.18 mmol) was dissolved in 2% 12 N HCl/EtOH (3.0 mL) and the mixture was stirred at rt for 20 min. Cold sat. $NaHCO_3$ was added to the mixture until it was basic (pH 8). The reaction was extracted with EtOAc. The organic layers were then washed with brine and dried over anhydrous sodium sulfate. Concentration in vacuo gave 73 mg (0.18 mmol, 100%) compound 482Ei as a yellow solid which was not purified further. HPLC: 95% at 1.73 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 min gradient with 0.2% H_3PO_4, monitoring at 220 nm). MS (ES): m/z 401.13 $[M+H]^+$.

Compound 482Dii (90 mg, 0.17 mmol) was dissolved in 2% 12 N HCl/EtOH (3.0 mL) and the mixture was stirred at rt for 20 min. Cold sat. $NaHCO_3$ was added to the mixture until it was basic (pH 8). The reaction was extracted with EtOAc. The organic layers were then washed with brine and dried over anhydrous sodium sulfate. Concentration in vacuo gave 70 mg (0.17 mmol, 100%) compound 482Eii as an orange solid which was not purified further. HPLC: 90% at 1.74 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 min gradient with 0.2% H_3PO_4, monitoring at 220 nm). MS (ES): m/z 401.14 $[M+H]^+$.

F. [3aR-(3aα,4β,5β,7β,7aα)]-7-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile (482F)

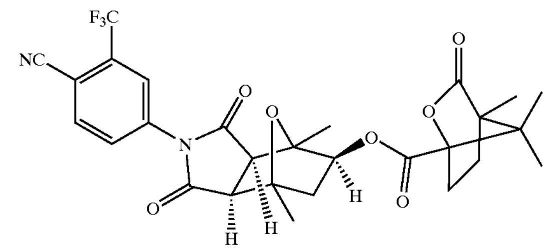
DBAD (21 mg, 0.090 mmol) was added to a solution of PPh_3 (24 mg, 0.090 mmol) in THF (0.4 mL). After stirring for 10 min, 5-chloro-2-pyridinol (12 mg, 0.090 mmol) was added and the reaction mixture was stirred for an additional 5 min. Compound 482Ei (18 mg, 0.045 mmol) was added and the mixture was stirred at rt for 1 h. The reaction was then concentrated under reduced pressure. Purification by preparative TLC eluting with 20% acetone/$CHCl_3$ gave 12 mg (0.023 mmol, 52%) of compound 482F. HPLC: 98% at 3.15 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 min gradient with 0.2% H_3PO_4, monitoring at 220 nm). MS (ES): m/z 512.11 $[M+H]^+$.

G. [3aS-(3aα,4β,5β,7β,7aα)]-7-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile (482G)

DBAD (21 mg, 0.090 mmol) was added to a solution of PPh_3 (24 mg, 0.090 mmol) in THF (0.4 mL). After stirring for 10 min, 5-chloro-2-pyridinol (12 mg, 0.090 mmol) was added and the reaction mixture was stirred for an additional 5 min. Compound 482Eii (18 mg, 0.045 mmol) was added and the mixture was stirred at rt for 1 h. The reaction was then concentrated under reduced pressure. Purification by preparative TLC eluting with 20% acetone/$CHCl_3$ gave 11 mg (0.021 mmol, 47.0%) of compound 482G. HPLC: 98% at 3.15 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 min gradient with 0.2% H_3PO_4, monitoring at 220 nm). MS (ES): m/z 512.15 $[M+H]^+$.

EXAMPLE 483

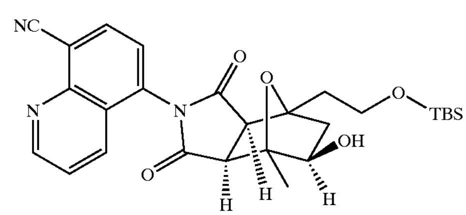
(1S,4R)-4,7,7-Trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxylic Acid, [3aS-(3aβ,4β,5β,7β,7aα)]-2-[4-cyano-3-(trifluoromethyl)phenyl]octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-1H-isoindol-5-yl Ester (483)



To a solution of compound 471Di (25 mg, 0.066 mmol) in 0.25 mL of CH_2Cl_2 at rt and under argon, was added a solution of (1S)-(−)-camphanic acid (20 mg, 0.10 mmol) in 0.2 mL of CH_2Cl_2. A solution of DCC (20 mg, 0.10 mmol) in 0.25 mL of CH_2Cl_2 was then added followed by DMAP (4.0 mg, 0.034 mmol). A white precipitate was obtained immediately and stirring was continued overnight. The precipitate was removed by filtration and the filtrate was diluted with EtOAc. The resulting solution was washed with 1N HCl, brine, sat. $NaHCO_3$, and brine then dried over $MgSO_4$. Concentration in vacuo afforded a viscous oily residue. The crude material was subjected to flash chromatography on a 20 cm^3 column of silica gel eluting with 50% EtOAc in hexanes to 32 mg of a white solid. Recrystallization from CH_2Cl_2/hexane yielded 20 mg (86%) of compound 483 as large crystals. This material was subjected to X-ray crystal diffraction studies to elucidate the exact stereochemistry of compound 471Di as referenced to the known fixed stereochemistry of the (1S)-(−)-camphanic acid appendage. LCMS: 100% at 1.9 min (retention time) (Phenomenex Luna C18 column, 2×30 mm, 0–100% aqueous acetonitrile over 3 min containing 10 mM NH_4OAc at 1 mL/min, monitoring at 220 nm). MS (EST): m/z 559.3 $[M-H]^-$.

EXAMPLE 484

(3aα,4β,5β,7β,7aα)-5-[7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (484)

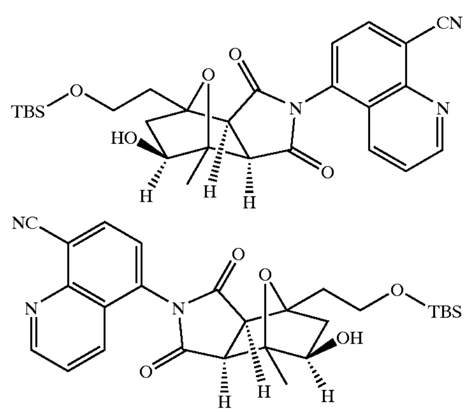


To a dry, 3-necked, 25 mL round-bottom was added $TiCl_2Cp_2$ (0.500 g, 2.01 mmol) and THF (4 mL) to give a deep red solution. Activated zinc dust (0.392 g, 6 mmol, prepared as described in Fieser and Fieser, Volume 1, p. 1276) was added and the suspension was stirred for 30 min during which time the color changed from brick-red to emerald-green. The unreacted zinc dust was allowed to settle. In a separate

3-necked, 25 mL round-bottom flask was added compound 464F (0.202 g, 0.399 mmol), THF (1 mL) and 1,4-cyclohexadiene (0.380 mL, 4.02 mmol). The Ti(III) reagent (0.90 mL, 0.45 mmol) was added via an addition funnel with a cotton plug at the bottom rinsing with THF (1 mL). After 1 h, HPLC showed ~50% conversion and an additional 0.9 mL (0.45 mmol) of the titanium reagent was added. After 1 h, HPLC showed complete consumption of starting material. Saturated ammonium chloride (5 mL) was added, followed by 10 mL of EtOAc. The organic layer was separated, washed with brine (5 mL), dried over Na_2SO_4, and concentrated in vacuo to give the crude product as an orange semi-solid. The crude material was purified by flash chromatography on silica gel eluting with 50% CH_2Cl_2/48% EtOAc/2% MeOH to give 0.10 g (59%) of compound 484 as a light yellow foam. HPLC: 91% at 3.65 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 508.27 $[M+H]^+$.

EXAMPLE 485

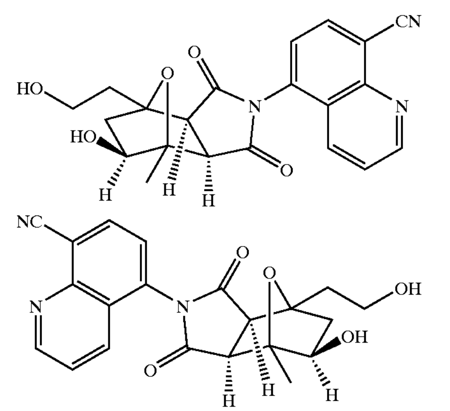
[3aR-(3aα,4β,5β,7β,7aα)]-5-[7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (485i) & [3aS-(3aα,4β,5β,7β,7aα)]-5-[7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (485ii)



The racemic compound 484, was separated into its individual antipodes by normal phase preparative chiral HPLC. A Chiralcel OD column (50×500 mm) was used with a flow rate of 50 mL/min (20% EtOH/hexanes) monitoring at 220 nm. The faster eluting antipode, compound 485i had a retention time of 35.8 min and the slower antipode, compound 485ii had a retention time of 49.7 min. Both antipodes were isolated as white solids after separation. Compound 485i: HPLC: 100% at 4.980 min (retention time) (Chiracel OD column (5×50 mm), 2.0 mL/min, 20% EtOH/hexanes, monitoring at 220 nm), >99% ee. MS (ES): m/z 508.23 $[M+H]^+$. Compound 485ii: HPLC: 98.6% at 7.357 min (retention time) (Chiracel OD column (5×50 mm), 2.0 mL/min, 20% EtOH/hexanes, monitoring at 220 nm), 97.2% ce. MS (ES): m/z 508.21 $[M+H]^+$. The absolute conformation for compounds 485i & 485ii was not established. For simplicity in nomenclature, compound 485i is designated herein as having an "R" configuration and compound 485ii as having an "S" configuration. Enantiomerically pure products derived from compound 4851 are designated herein as having a "R" configuration and enantiomerically pure products derived from compound 485ii are designated herein as having an "S" configuration.

EXAMPLE 486

[3aR-(3aα,4β,5β,7β,7aα)]-5-[Octahydro-5-hydroxy-7-(2-hydroxyethyl)-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (486i) & [3aS-(3aα,4β,5β,7β,7aα)]-5-[Octahydro-5-hydroxy-7-(2-hydroxyethyl)-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (486ii)



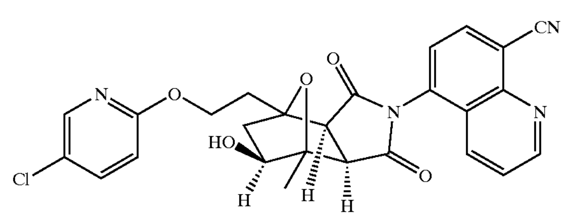
Compounds 485i & 485ii were converted to the free primary alcohol products as described in example 466 to give compounds 486i and 486ii as white solids.

Compound 486i: HPLC: 98% at 1.650 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 394.21 $[M+H]^+$.

Compound 486ii: HPLC: 98% at 1.663 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 394.20 $[M+H]^+$.

EXAMPLE 487

[3aR-(3aα,4β,7β,7aα)]-5-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (487)

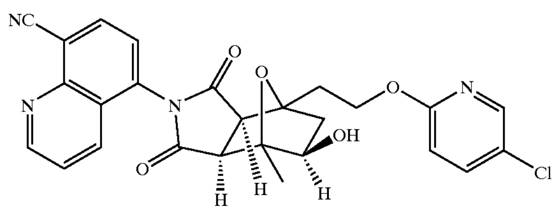


DBAD (0.088 g, 0.38 mmol) was added to a solution of triphenylphosphine (0.100 g, 0.382 mmol) in THF (1.0 m]L) at 22° C. and stirred for 10 min. 5-Chloro-2-pyridinol (0.049 g, 0.38 mmol) was added as a solid and stirring was continued for 10 min. The reaction mixture was added to compound 486i (0.100 g, 0.250 mmol) in THF (1.0 mL).

After stirring for 3 h, the reaction was concentrated in vacuo and purified by flash chromatography on silica gel eluting with 20–50% acetone/chloroform to give 0.080 g (63%) of compound 487 as a white solid. HPLC: 100% at 3.023 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 505.16 $[M+H]^+$.

EXAMPLE 488

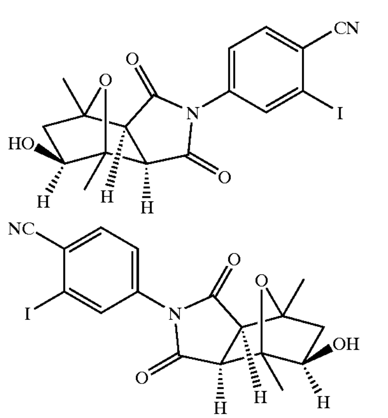
[3aS-(3aα,4β,7β,7aα)]-5-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (488)



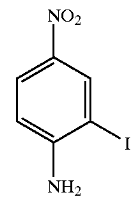
DBAD (0.088 g, 0.38 mmol) was added to a solution of triphenylphosphine (0.100 g, 0.382 mol) in THF (1.0 mL) at 22° C. and stirred for 10 min. 5-Chloro-2-pyridinol (0.049 g, 0.38 mmol) was added as a solid and stirring was continued for 10 min. The reaction mixture was added to compound 486ii (0.100 g, 0.250 mmol) in THF (1.0 mL). After stirring for 3 h, the reaction was concentrated in vacuo and purified by flash chromatography on silica gel eluting with 10–50% acetone/chloroform to give 0.080 g (63%) of compound 488 as a white solid. HPLC: 95% at 3.030 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 505.12 $[M+H]^+$.

EXAMPLE 489

[3aR-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-di methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile (489Gi) & [3aS-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile (489Gii)

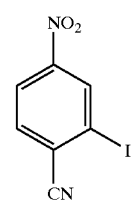


A. 2-Iodo-4-nitro-phenylamine (489A)



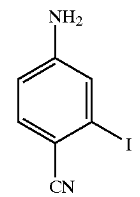
To a mixture of iodine (46.0 g, 0.180 mol) and silver sulfate (56.3 g, 0.180 mol) in anhydrous ethanol (500 mL) was added 4-nitroaniline (25.0 g, 0.180 mol) and the reaction mixture was stirred for 5 h at rt. The resulting yellow solution was filtered and concentrated in vacuo. The resulting residue was dissolved into 400 mL ethyl acetate, washed with 1N sodium hydroxide solution (2×250 mL), dried over sodium sulfate, filtered, and concentrated in vacuo to yield 45.5 (95%) of compound 489A, as a yellow solid. HPLC: 98% at 2.837 min (retention time) (Shimadzu VP-ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% trifluoracetic acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 265.08 $[M+H]^+$.

B. 2-Iodo-4-nitro-benzonitrile (489B)



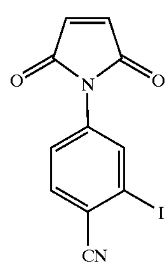
Compound 489A (10.0 g, 37.9 mmol) was dissolved in a mixture of 20 mL 12 N HCl/40 mL water and then cooled to 0° C. To this mixture was slowly added a solution of sodium nitrite (5.23 g, 75.8 mmol) in 10 mL water while maintaining the reaction temperature at 0° C. The reaction was stirred for 1 h at 0° C. and then slowly added to a mechanically stirred solution of freshly prepared cuprous cyanide (3.0 g, 33 mmol, prepared as described in Vogel's Textbook of Practical Organic Chemistry, 5th edition, pg. 429) and potassium cyanide (6.30 g, 96.7 mmol) in water (50 mL) at 50° C. The reaction was stirred for 1 h at 50° C., cooled to 25° C. and extracted with methylene chloride (2×200 mL). The organic portion was dried over sodium sulfate, filtered and concentrated in vacuo. The resulting residue was purified by chromatography on silica gel eluting with 4:1 hexane:ethyl acetate to yield 4.6 g (44%) of compound 489B as an orange solid. HPLC: 98% at 2.647 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

C. 4-Amino-2-iodo-benzonitrile (489C)



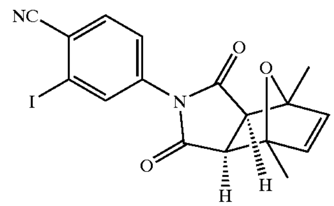
A mixture of compound 489B (4.60 g, 16.8 mmol), tetrahydrofuran (75 mL), ethanol (100 mL), ammonium chloride solution (1.51 g, 28.3 mmol, dissolved in 100 mL of water), and iron (325 mesh, 4.21 g, 75.4 mmol) was mechanically stirred. The reaction mixture was heated to reflux for 3 h or until all starting material was consumed. The reaction mixture was cooled, filtered through Celite and concentrated in vacuo. The resulting residue was dissolved in ethyl acetate (200 mL) and washed with 1N sodium hydroxide (2×150 mL), dried over sodium sulfate, filtered and concentrated in vacuo to yield 3.97 g (97%) of compound 489C as a dark solid. HPLC: 95% at 1.877 min (retention lime) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 245.13 $[M+H]^+$.

D. 4-(2,5-Dioxo-2,5-dihydro-pyrrol-1-yl)-2-iodo-benzonitrile (489D)



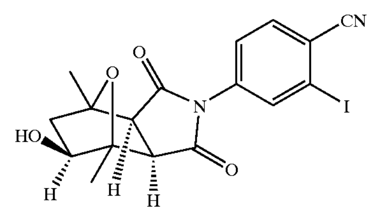
Compound 489C (3.97 g, 16.3 mmol) and maleic anhydride (2.41 g, 24.4 mmol) were refluxed in glacial acetic acid (15 mL) for 5 h. The reaction was cooled to 25° C. and then poured onto ice (100 mL). The resulting precipitate was isolated by filtration and washed with water (2×25 mL) and dried under vacuum to yield 4.78 g (90%) of compound 489D as a tan solid. HPLC: 82% at 2.68 min (retention time) C Shimadzu VP-ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% trifluoroacetic acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 325.04 $[M+H]^+$.

E. (3aα,4β,7β,7aα)-4-(1,3,3a,4,7,7a-Hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile (489E)



A solution of compound 489D (0.40 g, 1.2 mmol) in 2,5-dimethylfuran (2.0 g) was stirred at 75° C. for 2 h. The reaction was decanted from any insoluble materials and the particulates were washed with diethyl ether. The combined decant and ether washes were combined and concentrated in vacuo while maintaining a temperature of <50° C. The resulting residue was triturated with hexanes to yield 0.56 g (94% based on purity) of compound 489E as a tan solid. Due to the propensity of the product to undergo a retro-Diels-Alder reaction, no further purification was attempted. HPLC: 85% at 3.01 min (retention time) (Shimadzu VP-ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% trifluoroacetic acid acid, 4 mL/min, monitoring at 254 nm). MS (ES): m/z 421.05$[M+H]^+$.

F. (3aα,4β,5β,7β,7aα)-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile (489F)



To a solution of compound 489E (0.40 g, 0.95 mmol) in dry THF (5 mL) cooled to 0° C. was added borane-dimethylsulfide complex (0.2 mL, 1.9 mmol, 10 M) and the reaction solution was allowed to warm to 25° C. After stirring for 30 min, the reaction was cooled to 0° C. and pH 7 phosphate buffer (6.6 mL) was slowly added, followed by the addition of 30% hydrogen peroxide (0.7 mL). The reaction was stirred at 25° C. for 1 h and then partitioned between ethyl acetate (100 mL) and water (100 mL). The organic portion was isolated, dried over sodium sulfate, filtered and concentrated in vacuo. The resulting residue was purified by silica gel eluting with 3:1 methylene chloride-:ethyl acetate to yield 0.11 g (25%) of compound 489F as a white solid. HPLC: 99% at 2.527 min (retention time) (Shimadzu VP-ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% trifluoroacetic acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 439.09 $[M+H]^+$.

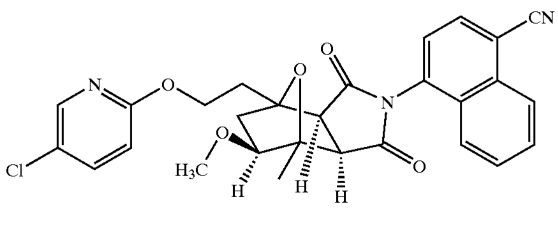
G. [3aR-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile (489Gi) & [3aS-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-iodobenzonitrile (489Gii)

The racemic compounds 489F, was separated into its individual antipodes by normal phase preparative chiral HPLC. A Chiralcel AD column (50×500 mm) was used with a flow rate of 50 mL/min (70% Isopropanol/hexanes) monitoring at 220 nm. The faster eluting antipode, compound 489Gi had a retention time of 4.587 min and the slower eluting antipode, compound 489Gii had a retention time of 6.496 min. Both antipodes were isolated as white solids after separation. The absolute conformation for compounds

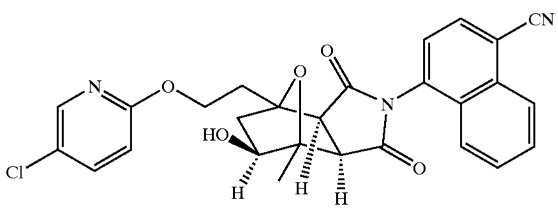
489Gi & 489Gii was not established. For simplicity in nomenclature, compound 489Gi is designated herein as having an "R" configuration and compound 489Gii as having an "S" configuration.

EXAMPLE 490

(3aα,4β,5β,7β,7aα)-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-methoxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (490B)



A. (3aα,4β,7β,7aα)-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (490A)



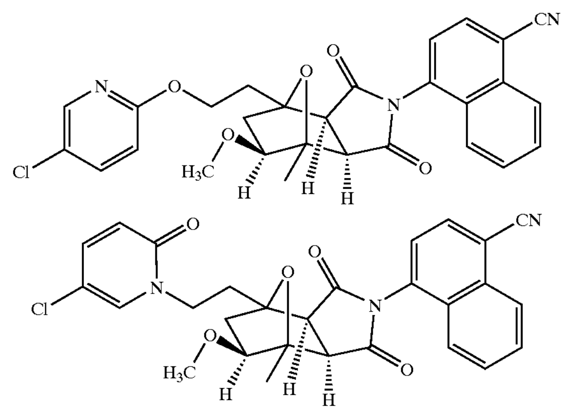
A mixture of triphenylphosphine (166 mg, 0.633 mmol) and DBAD (146 mg, 0.633 mmol) was dissolved in THF (4 mL) under nitrogen and the yellow solution was stirred for 10 min. 5-Chloro-pyridin-2-ol (82 mg, 0.63 mmol) was added and the mixture was stirred for 5 min after which compound 242B (165 mg, 0.327 mmol) was added. The mixture was stirred for 12 h and the solvent was removed under a stream of nitrogen. The resulting oil was adsorb onto silica gel (1 g) and purified by flash chromatography on a Jones Chromatography silica cartridge (5 g/25 mL) eluting with a gradient of 0–50% acetone in chloroform to give 79.4 mg (47%) of compound 490A as a white foam. HPLC: 99% at 3.48 min (retention time) (Phenomenex ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 504.17 $[M+H]^+$.

B. (3aα,4β,5β,7β,7aα)-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-methoxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (490B)

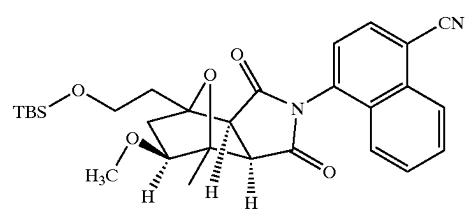
Compound 490A (24 mg, 0.048 mmol) was dried into a 1 dram vial with a magnetic stir bar. Silver oxide (57 mg, 0.24 mmol), CH_3CN (500 μL) and iodomethane (20 μL, 0.32 mmol) were added under nitrogen and the mixture was put in a heated block (82° C.) and stirred for 14 h. The mixture turned brown after 20 min then green. The mixture was filtered through Celite and Florisil and was purified by reverse phase preparative HPLC (Shimadzu Shimpac VP ODS column, 20×50 mm, 0–100% aqueous methanol over 6 min containing 0.1% TFA, monitoring at 220 nm) to give 6.9 mg (28%) of compound 490B as a white foam. HPLC: 99% at 3.64 min, 3.76 min (atropisomers, retention time) (Phenomenex ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 518.19 $[M+H]^+$.

EXAMPLE 491

[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-methoxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, (491Ci) & [3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(5-Chloro-2-oxo-1(2H)-pyridinyl)ethyl]octahydro-5-methoxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (491Cii)

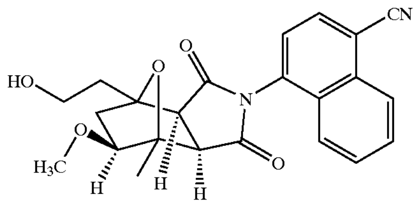


A. (3aα,4β,5β,7β,7aα)-4-[7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]octahydro-5-methoxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (491A)



Compound 243Cii (142 mg, 0.280 mmol) was dried into a 1 dram vial equipped with a magnetic stir-bar and a Teflon lined cap. Silver oxide (324 mg, 1.40 mmol), CH_3CN (3 mL) and iodomethane (90 μL, 1.4 mmol) were added under nitrogen and the mixture was put in a heated block (82° C.). The reaction was stirred overnight, then filtered through Celite and concentrated in vacuo. The resulting residue was purified by flash chromatography on silica gel eluting with a gradient of 0–50% acetone in chloroform to yield 62.2 mg (43%) of compound 491A. HPLC: 99% at 3.87 & 3.95 min (atropisomers, retention time) (Phenomenex ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 521.37 $[M+H]^+$.

B. 3aα,4β,5β,7β,7aα)-4-[Octahydro-7-(2-hydroxyethyl)-5-methoxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (491B)



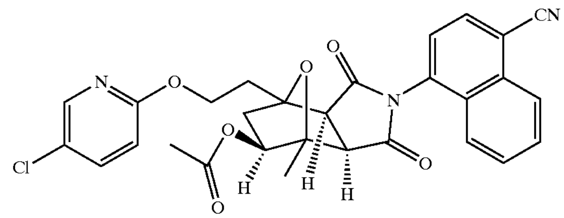
Compound 491A (62.2 mg, 0.119 mmol) was dissolved in ethanol (2 mL) and 12 N hydrochloric acid (50 μL) was added and the mixture was stirred for 10 min. The solvent was removed in vacuo and the product was purified by flash chromatography on silica gel eluting with a gradient of 0–20% acetone in chloroform to yield 40.3 mg (83%) of compound 491B as a white solid. HPLC: 96% at 2.30 & 2.45 min (atropisomers, retention time) (Phenomenex ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 407.22 $[M+H]^+$.

C. [3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-methoxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, Slow Eluting Enantiomer (491Ci) & [3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(5-Chloro-2-oxo-1(2H)-pyridinyl)ethyl]octahydro-5-methoxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (491Cii)

Triphenylphosphine (40 mg, 0.15 mmol) and DBAD (35 mg, 0.15 mmol) were dissolved in THF under nitrogen and stirred 10 min. 5-Chloropyridin-2-ol (20 mg, 0.15 mmol) was added and the mixture was stirred for 5 min. Compound 491B (40.3 mg, 0.0991 mmol) was added and the resulting mixture was stirred for 2.5 h. The solvent was concentrated in vacuo and the resulting residue was purified by chromatography over Florisil (1.3 g) eluting with a gradient of 0–40% acetone in chloroform to give 140 mg of a mixture of 491Ci, 491Cii and DBAD. The oil was suspended in dichloromethane (3 mL) and trifluoroacetic acid (2 mL) was added. After 45 min, the solvent was removed in vacuo and the resulting oil was partitioned between saturated sodium bicarbonate (20 mL) and EtOAc (20 mL). The aqueous layer was extracted with EtOAc (2×20 mL) and the combined organic layers were dried over magnesium sulfate. Purification by reverse phase preparative HPLC (Shimadzu Shimpac VP ODS column, 20×50 mm, 0–100% aqueous methanol over 6 min containing 0.1% TFA, monitoring at 220 nm) gave 22 mg (44%) of compound 491Ci and 4.6 mg (9% yield) of compound 491Cii. Compound 491Ci: HPLC: 95% at 3.50 min (retention time) (Phenomenex ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 518.28 $[M+H]^+$. Compound 491Cii: HPLC: 85% at 2.94 & 3.07 min (atropisomers, retention time) (Phenomenex ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 518.27 $[M+H]^+$.

EXAMPLE 492

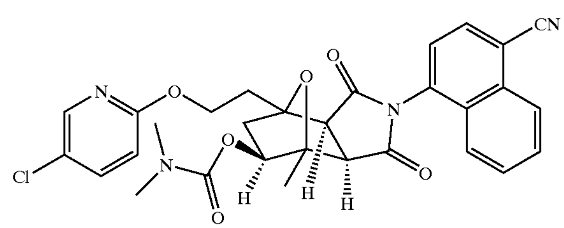
[3aR-(3aα,4β,5β,7β,7aα)]-4-[5-(Acetyloxy)-7-[2-[(5-chloro-2-pyridinyl)oxy]ethyl]octahydro-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, Slow Eluting Enantiomer (492)



Acetyl chloride (25 μL, 0.35 mmol) was added to a solution of compound 490A (30 mg, 0.060 mmol) in pyridine (600 μL). The mixture was stirred overnight, diluted with hydrochloric acid (0.5 N, 10 mL), extracted with chloroform (3×7 mL). The organic layers were combined, washed with water (3×4 mL) and brine (4 mL), dried over magnesium sulfate and concentrated in vacuo. Purification by flash chromatography on silica gel eluting with a gradient of 0–50% acetone in chloroform gave 17 mg (53%) of compound 492. HPLC: 99% at 3.48 & 3.63 min (atropisomers, retention time) (Phenomenex ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 546.15 $[M+H]^+$.

EXAMPLE 493

Dimethylcarbamic Acid, [3aR-(3aα,4β,5β,7β,7aα)]-7-[2-[(5-chloro-2-pyridinyl)oxy]ethyl]-2-(4-cyano-1-naphthalenyl)octahydro-4-methyl-1,3-dioxo-4,7-epoxy-1H-isoindol-5-yl ester, Slow Eluting Enantiomer (493)

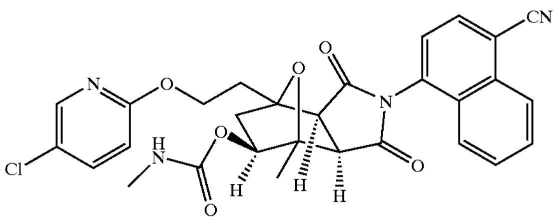


To a solution of compound 490A (30 mg, 0.060 mmol) in pyridine (300 μl) was added dimethylcarbamyl chloride (28 μl, 0.30 mmol) and the mixture was stirred at 25° C. for 12 h. An additional portion of dimethylcarbamyl chloride (28 μL, 0.30 mmol) was added and the reaction was heated at 70° C. for 12 h. A third portion of dimethylcarbamoyl chloride (28 μl, 0.30 mmol) as well as pyridine (300 μl) were added and the mixture was stirred at 100° C. for 24 h. The solution was diluted with 0.5 N HCl (10 mL) and extracted with chloroform (3×7 mL). The organic layers were combined and washed with water (3×4 mL) and brine (4 mL), dried over magnesium sulfate and concentrated in vacuo. Purification by reverse phase preparative HPLC (Shimadzu Shimpac VP ODS column, 20×50 mm, 0–100% aqueous methanol over 6 min containing 0.1% TFA, monitoring at 220 nm) gave 15.7 mg (46%) of compound 493 as a white

solid. HPLC: 99% at 3.52 min & 3.69 min (atropisomers, retention time) (Phenomenex ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 575.10 $[M+H]^+$.

EXAMPLE 494

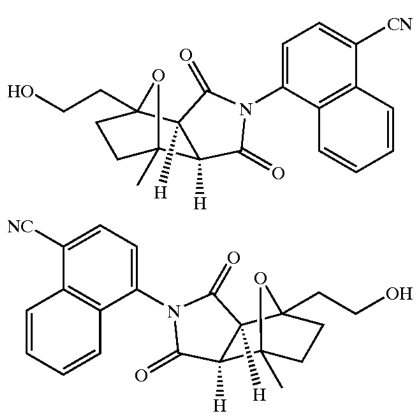
[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-4-methyl-5-[[(methylamino)carbonyl]oxy]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, Slow Eluting Enantiomer (494)



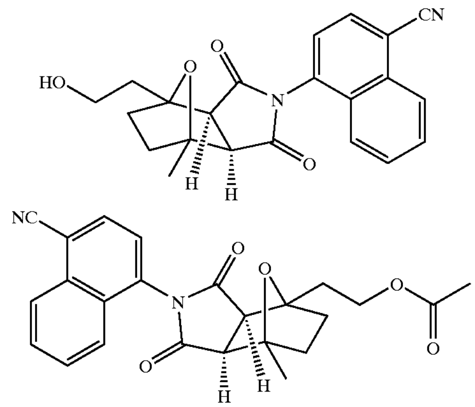
Methyl isocyanate (36 μL, 0.60 mmol) was added to a solution of compound 490A (30 mg, 0.060 mmol) in dioxane (600 μL) and was heated at 80° C. overnight. An additional portion of methyl isocyanate (36 μL, 0.60 mmol) was added and the mixture was heated at 100° C. for 24 h. A third portion of methyl isocyanate (36 μL, 0.60 mmol) was added and the mixture was stirred at 100° C. for 24 h. The solvent was removed in vacuo and the oil was purified by reverse phase preparative HPLC (Shimadzu Shimpac VP ODS column, 20×50 mm, 0–100% aqueous methanol over 6 min containing 0.1% TFA, monitoring at 220 nm) to give 20 mg (59%) of compound 494 as a clear glass. HPLC: 99% at 3.33 min & 3.42 min (atropisomers, retention time) (Phenomenex ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 561.08 $[M+H]^+$.

EXAMPLE 495

[3aR(3aα,4β,5β,7β,7aα)]4-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (495Ai) & [3aS(3aα,4β,5β,7β,7aα)]-4-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (495B)



A. [3aS-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (495Ai) & (3aα,4β,5β,7β,7aα)-4-[2-(Acetyloxy)ethyl]-2-(4-cyano-1-naphthalenyl)hexahydro-7-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione (495Aii)



Racemic compound 223B (10.26 g, 27.26 mmol) was dissolved in anhydrous THF (500 mL) in a 10 L bottle. tert-Butyl methyl ether (4.86 L), vinyl acetate (216 mL) and Lipase (108 g, [Sigma, Lipase type II, crude from Porcine pancreas, product No. L3126, Lot No. 021K1445]) were added. The reaction mixture was agitated for 24 h at rt and the reaction was monitored by HPLC using the following conditions: A 200 μL sample of the reaction mixture was filtered, dried under a stream of nitrogen and subjected to HPLC analysis (YMC ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). The reaction was stopped after 60% of the starting material was consumed. The enzyme was removed by filtration and the filtrate was concentrated it vacuo. The resulting residue was dissolved in $CHCl_3$ and absorbed onto silica gel. Purification by flash chromatography on silica gel eluting with a gradient of 1–5% MeOH in $CHCl_3$ gave 3.78 g (37%) of compound 495Ai and 6.84 g (60%) of compound 495Aii, both as white solids. Compound 495Ai: HPLC: 99% at 3.47 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% TFA, 4 mL/min, monitoring at 220 mm). MS (ES): m/z 377.09 $[M+H]^+$. Normal phase preparative chiral HPLC: 37.8 min (retention time) (chiralpak AD column, 4.6×250 mm, 10 micron, 40° C., isocratic elution with 8% EtOH/MeOH (1:1) in heptane, monitoring at 220 nm), 99% ee. Compound 495Aii: HPLC: 99% at 2.92 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% TFA, 4 mL/min, monitoring at 220 nm).

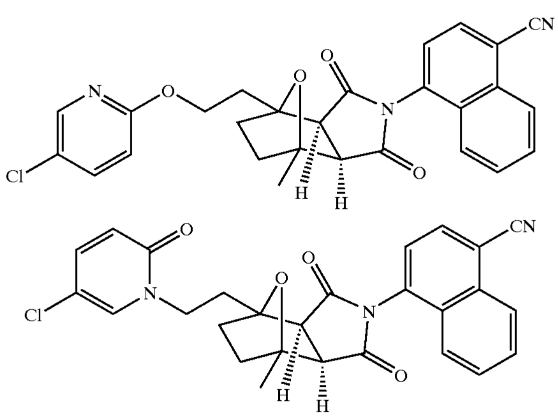
B. [3aR-(3aα,4β,5β,7β,7aα)]4-[Octahydro-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (495B)

Lipase (134 g, [Sigma, Lipase type II, crude from Porcine pancreas, product No. L3126, Lot No. 021K1445]) was added to 3.5 L of deionized water. The mixture was centrifuged to remove most of the suspended material. The pH of the supernatant was adjusted to 7.06 with 1N sodium hydroxide and a solution of compound 495Aii (8.04 g, 19.2 mmol) in TBME (1.5 L) was added. The pH was increased to 7.16 by addition of 1N sodium hydroxide. The reaction

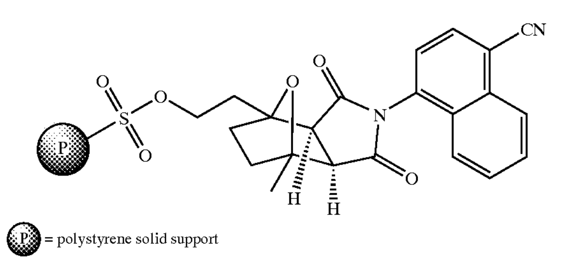
mixture was agitated at rt and was monitored by analytical HPLC as described in Example 495A. After 30 min, the reaction was filtered through Celite and the filtrate was extracted with ethyl acetate (4×1 L) until HPLC showed that all the alcohol has been removed. The organic fractions were combined, dried over magnesium sulfate, filtered and concentrated in vacuo. Purification by flash chromatography on silica gel (Jones, 50 g column) using a gradient of 0–70% acetone in chloroform followed by 5% MeOH in chloroform gave 2.44 g (33%) of compound 495B as a white solid. HPLC: 99% at 2.89 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 377.09 $[M+H]^+$. Normal phase preparative chiral HPLC: 11.1 min (retention time) (chiralpak AD column, 4.6×250 mm, 10 micron, 40° C., isocratic elution with 8% EtOH/MeOH (1:1) in heptane, monitoring at 220 nm), 95% ee.

EXAMPLE 496

[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (496Bi) & [3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(5-Chloro-2-oxo-1(2H)-pyridinyl)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (496Bii)



A. Preparation of Solid Support (496A)



A mixture of anhydrous CH_2Cl_2 (10 mL) and pyridine (10 mL) was added under nitrogen to chlorosulfonylpolystyrene (Argonaut, 1.70 mmol/g, 3.0 g, 5.1 mmol) and compound 495Ai (3.78 g, 10.0 mmol) in a polymer synthesis tube. The mixture turned into a yellow gel and was vigorously shaken (wrist action shaker for 4 h.). All the solvents were absorbed by the resin and it looked dry. The resin was washed in portions with CH_2Cl_2 (200 mL) and the washes were combined and extracted with 200 ml 1N HCl. The HCl fraction was re-extracted with ethyl acetate (200 mL). The organic fractions were combined and extracted with water (50 mL), brine (50 mL) and the organic fractions were dried over sodium sulfate, filtered and concentrated to give 2.1 g of resin bound compound 496A (89% loaded based on recovered unbound compound 495Ai). The resin was washed consecutively with DMF (5×), DMF:water (3:1, 5×), THF (3×) and CH_2Cl_2 (3×) (~30 mL each wash). The resin was dried in vacuo for 1 h to yield 5.35 g of resin. The resin was still wet and was re-treated with the alcohol. The above described loading process was repeated using the recovered un-reacted compound 495Ai from the above procedure. Recovered compound 495Ai (2.1 g, 5.6 mmol) and anhydrous dichloromethane (15 mL) and pyridine (15 mL) and the resin were combined and subjected to the reaction conditions described above. The resulting resin was washed as described previously and dried in vacuo overnight to yield 4.49 g of resin (87% loaded based on recovered unbound compound 495Ai). The starting alcohol was recovered from the dichloromethane:pyridine mixture as described previously (2.04 g of white solid, which would suggest a 92% loading based on recovered alcohol). The resin weigh increase is usually more accurate for loading assessment. The resin loading was calculated to be 0.87 (determined by resin weight increase)×1.08 mmol/g (calculated 100% loading)=0.94 mmol/g. The resin was used as is for the next step.

B. [3aR-(3aα,4β,7β,7aα)]-4-[4-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (496Bi) & [3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(5-Chloro-2-oxo-1 (2H)-pyridinyl)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (496Bii)

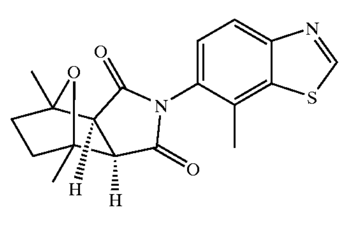
The following procedure describes a general process by which arrays of compounds of Formula I can be made using automated approaches. Additional information on such automated synthetic approaches can be found in Example 8. Compounds 496Bi & 496Bii are an example of compounds made by such a procedure. For compounds 496Bi & 496Bii, 4-chloropyridinol represents the nuceophile reagent. A broader definition of the term nucleophile is contained in the body of this document and is well understood by one skilled in the art. A Bohdan MiniReactor equipped with a heating/cooling block was used with 0.5 Dram vials stacked over one another to achieve the same level as the reactor tubes. Resin (compound 496A) was measured into the individual vials by using each Bohdan resin Transfer module plate "10 mg" and "20 mg" once. The weights of resin delivered ranged from 17–23 mg (0.016–0.022 mmol). Cesium carbonate was added using the Bohdan "20 mg" plate which delivered ~57–60 mg (0.17–0.18 mmol). The nucleophiles were weighted into 1 Dram vials and were diluted in THF to 0.06 M using a Tecan eight channel liquid handler. The resulting solutions (250 μL, 0.015 mmol) were added manually via a micro-pipette to each of the reaction vials containing resin and the resulting array of reaction vials were placed in a Bohdan reactor. When the nucleophiles are

amines, ~13 μL of diisopropylethylamine was added to the THF solution of the amine. The vials were capped (Teflon-lined) and the reactions were heated with orbital shaking (short stroke 500 rpm) at 70° C. for 24 h. The reactions were cooled to 25° C. and 1 mL of a mixture of heptane and ethyl acetate (1:1) was added followed by 0.5 mL of water. The organic layer was extracted manually and individually transferred to a synthesis block tube containing magnesium sulfate (~150 mg). The array of synthesis block tubes were simultaneously filtered and the filtrates were individually collected into microtubes (96 well block). The aqueous layer was re-extracted with 1 mL of a mixture of heptane and ethyl acetate (1:1), the organic layer was filtered as described above and the filtrate was individually collected as described above into the existing microtubes.

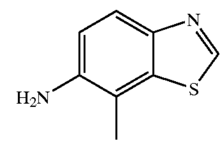
Analysis of the array of compounds prepared by the above procedure was performed using the following automated approach. A 120 μl portion of each of the above reaction (filtrates) was aliquoted into two 96 deep well blocks for analysis. The solvent was concentrated in vacuo and the plates were re-diluted with methanol (500 μL). One plate was analyzed by LCMS (Phenomenex ODS column, 4.6×50 mm, 4 mL/min, gradient 0% A to 100% B (A: 90% water, 10% MeOH, 0.1% TFA; B: A: 90% MeOH, 10% water, 0.1% TFA) and the other by flow-NMR (Varian Inova-500 MHz, MeOH, WET solvent suppression pulse sequence, 128 scans, 60 μl flow cell probe). The criteria for submission was: correct molecular ion present and HPLC/NMR purity >70%. Compounds which did not meet the desired criteria were purified by reverse phase preparative HPLC (Shimadzu UP-ODS column, 20×50 mm, 20 mL/min, gradient 40% B to 100% B in 6 min with 2 min hold (A: 90% water, 10% MeOH, 0.1% TFA; B: A: 90% MeOH, 10% water, 0.1% TFA). HPLC purification yielded 3.4 mg (21%) of compound 496Bi as a glassy solid and 6.8 mg (41%) of compound 496Bii as a glassy solid. Compound 496Bi: HPLC: 96% at 3.47 min & 3.62 min (atropisomers, retention time) (Phenominex ODS column, 4.6×50 mm, 4 mL/min, gradient 0% A to 100% B (A: 90% water, 10% MeOH, 0.1% TFA; B: A: 90% MeOH, 10% water, 0.1% TFA), monitoring at 220 nm). MS (ES): m/z 487.94 $[M+H]^+$. Compound 496Bii: HPLC: 96% at 3.00 min & 3.12 min (atropisomers, retention time) (Phenominex ODS column, 4.6×50 mm, 4 mL/min, gradient 0% A to 100% B (A: 90% water, 10% MeOH, 0.1% TFA; B: A: 90% MeOH, 10% water, 0.1% TFA), monitoring at 220 nm). MS (ES): m/z 488.12 $[M+H]^+$. Additional compounds made by this procedure are set forth in Table 17.

EXAMPLE 497

(3aα,4β,5β,7β,7aα)-Hexahydro-4,7-dimethyl-2-(7-methyl-6-benzothiazolyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione (497B)



A. 6-Amino-7-methylbenzothiazole (497A)



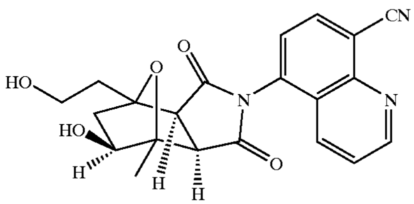
7-Methyl-6-nitrosobenzothiazole was prepared from 6-nitrobenzothiazole according to the general procedure described by Bartoli et al. *Synlett* 270 (1976). To a solution of 7-methyl-6-nitrosobenzothiazole (889 mg, 5.00 mmol) in AcOH (40 mL) at 70° C. was added iron powder (325 mesh, 559 mg, 10.0 mmol) in a single portion. The resulting dark reaction mixture was stirred for 15 min before it was cooled and concentrated in vacuo to leave a residue which was partitioned between 1N HCl (50 mL) and CH_2Cl_2 (50 mL). The layers were separated and the organic layer was washed once with 1N HCl (25 mL). The combined aqueous layers were made basic by the addition of solid $NaHCO_3$ and were extracted twice with EtOAc. The organic phases were combined, dried over $MgSO_4$ and concentrated in vacuo to give 534 mg (65%) of compound 497A as a light brown solid. HPLC: 96% at 0.55 min (retention time) (YMC S5 ODS column, 4.6×50 mm Ballistic, 10–90% aqueous methanol over 4 min containing 0.2% H_3PO_4, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 165.0 $[M+H]^+$.

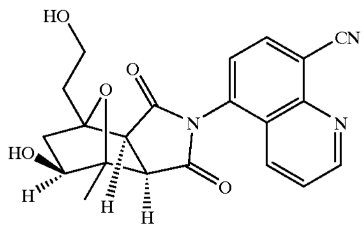
B. (3aα,4β,5β,7β,7aα)-Hexahydro-4,7-dimethyl-2-(7-methyl-6-benzothiazolyl)-4,7-epoxy-1H-isoindole-1,3(2H)-dione (497B)

6-Amino-7-methylbenzothiazole (29 mg, 0.18 mmol), $MgSO_4$ (54 mg, 0.45 mmol), triethylamine (125 μL, 0.897 mmol) and compound 20A (52 mg, 0.26 mmol) were taken up in 0.18 mL of DME and placed in a sealed tube. The sealed tube was heated at 135° C. for 14 h. The cooled reaction mixture was filtered through a short pad of Celite eluting with EtOAc and the solvent was removed in vacuo. The residue was purified by reverse phase preparative HPLC (YMC S5 ODS column, 20×100 mm, eluting with 30–100% aqueous methanol over 10 min containing 0.1% TFA, 20 mL/min, monitoring at 220 nm). Concentration of the desired fractions afforded a residue which was partitioned between CH_2Cl_2 (10 mL) and sat. $NaHCO_3$ solution (10 mL). The aqueous layer was extracted once with CH_2Cl_2 and the combined organic phases were dried over Na_2SO_4 and concentrated in vacuo to give 42 mg (68%) of compound 497B as a tan solid. HPLC: 2.36 min & 2.55 min (atropisomers, retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 343.3 $[M+H]^+$.

EXAMPLE 498

(3aα,4β,5β,7β,7aα)-5-[Octahydro-5-hydroxy-7-(2-hydroxyethyl)-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (498i) & (3aα,4β,5β,7β,7aα)-5-[Octahydro-5-hydroxy-4-(2-hydroxyethyl)-7-methyl-1,3-dioxo-4,7-enoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile (498ii)



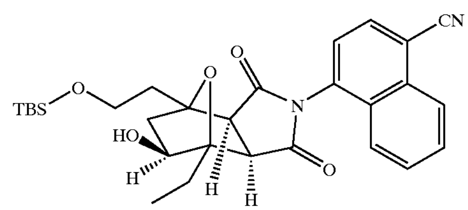


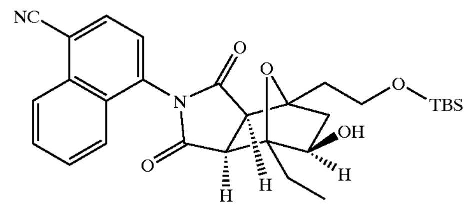
Compound 464E (0.500 g, 1.02 mmol) was dissolved in THF (5.00 mL) and cooled to 0° C. BH_3.DMS (0.193 mL, 2.04 mmol) was then added slowly followed by warming to 25° C. After 1 h, the reaction was cooled to 0° C. and pH 7 phosphate buffer (15.0 mL) was added resulting in the evolution of gas. EtOH (7.0 mL) and hydrogen peroxide (30%, 1.5 mL) were then added and the reaction was warmed to 25° C. over 2 h. After 3 h, the mixture was extracted with methylene chloride (3×50 mL). The combined organic layers were washed once with brine and dried over anhydrous sodium sulfate. The product was complexed to boron after workup. All attempts to break up this complex failed to give the free product. The crude material was taken on to the next step without further purification.

The crude reaction mixture was dissolved in 2% conc. HCl/MeOH (5.0 mL) at rt. After 1 h, the volatiles were removed in vacuo and the resulting residue was dissolved in methylene chloride and washed once with sat. aq. sodium bicarbonate and dried over anhydrous sodium sulfate. Solvent removal in vacuo gave the crude mixture of compounds 498i and 498ii as a yellow solid. The mixture of compounds was separated by reverse phase preparative HPLC: Compound 498i: 17.994 min (retention time) & compound 498ii: 19.767 min (retention time) (YMC S5 ODS column, 30×250 mm, 25 mL/min, 10–90% aqueous methanol over 35 min containing 0.1% TFA, monitoring at 220 nm). Solvent removal in vacuo gave 0.012 g (3%) of compound 498i as a white solid and 0.009 g (2%) of compound 498ii as a white solid. Compound 498i: HPLC: 85% at 1.843 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 394.21 $[M+H]^+$. Compound 498ii: HPLC: 98% at 1.650 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 394.21 $[M+H]^+$.

EXAMPLE 499

[3aR-(3aα4β,5β,7β,7aα)]-4-[7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-4-ethyloctahydro-5-hydroxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (499i) & [3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-4-ethyloctahydro-5-hydroxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (499ii)

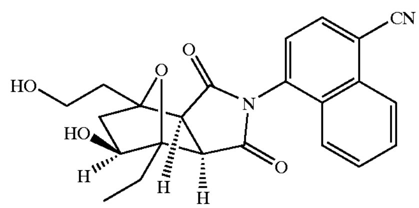


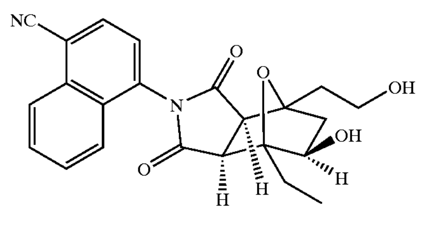


The racemic compound 434C was separated into its individual antipodes by normal phase preparative chiral HPLC using a Chiracel AD column (5 cm×50 cm), eluting with 8% EtOH in hexane at 50 mL/min to give the faster eluting compound 499i (Chiral HPLC: 6.74 min; CHIRALCEL AD 4.6×250 mm column; isocratic elution with 10% EtOH in hexane at 2 mL/min) and the slower eluting compound 499ii (Chiral HPLC: 9.99 min; CHIRALCEL AD 4.6×250 mm column; isocratic elution with 10% EtOH in hexane at 2 mL/min). For either compound 499i or 499ii: HPLC: 100% at 3.96 min (retention time) (YMC CombiScreen ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 4 min, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 521.25 $[M+H]^+$. The absolute stereochemistry for compounds 499i & 499ii was not established. For simplicity in nomenclature, compound 499i is designated herein as having an "R" configuration and compound 499ii as having an "S" configuration. Enantiomerically pure products derived from compound 4991 are designated herein as having a "R" configuration and enantiomerically pure products derived from compound 499ii are designated herein as having an "S" configuration.

EXAMPLE 500

[3aR-(3aα,4β,5β,7β,7aα)]-4-[4-Ethyloctahydro-5-hydroxy-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (500i) & [3aS-(3aα,4β,5β,7β,7aα)]-4-[4-Ethyloctahydro-5-hydroxy-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (500ii)



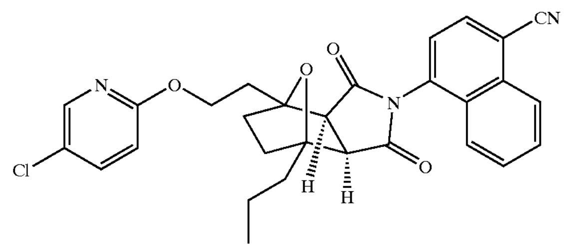


Compound 499i (24.0 mg, 0.0461 mmol) was dissolved in 2% conc. HCl/EtOH (0.8 mL) and the mixture was stirred at rt for 20 min. Cold sat. $NaHCO_3$ was added to the mixture until the solution reached pH 8, then extracted with EtOAc. The organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. Concentration in vacuo gave 14.7 mg (78%) of compound 500i as a white solid which did not require further purification. HPLC: 95% at 2.40 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 min gradient with 0.2% H_3PO_4, monitoring at 220 nm).

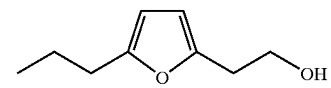
Compound 499ii (18.0 mg, 0.0346 mmol) was dissolved in 2% conc. HCl/EtOH (0.6 mL) and the mixture was stirred at rt for 20 min. Cold sat. $NaHCO_3$ was added to the mixture until the solution reached pH 8, then extracted with EtOAc. The organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. Concentration in vacuo gave 14.1 mg (99%) of compound 500ii as a white solid which did not require further purification. HPLC: 95% at 2.40 min (retention time) (YMC S5 ODS 4.6×50 mm, 10%–90% aqueous methanol over 4 min gradient with 0.2% H_3PO_4, monitoring at 220 nm).

EXAMPLE 501

[3aR-(3α,4β,7β,7aα)]-4-[4-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-1,3-dioxo-7-propyl-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (501D)

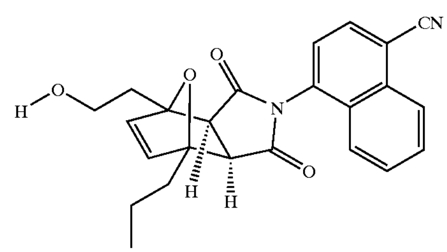


A. 2-(5-Propyl-furan-2-yl)-ethanol (501A)



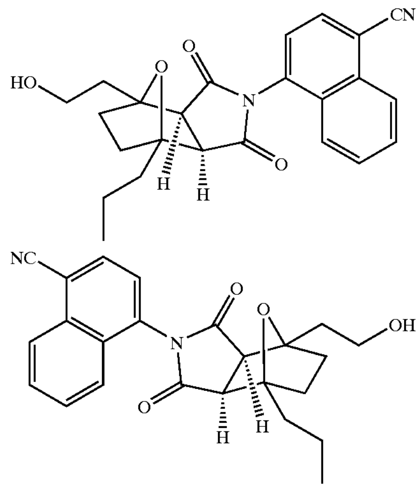
To a solution of 2-propylfuran (3.00 g, 31.2 mmol) in THF (31 mL) at −78° C. was added n-BuLi (15.0 mL, 2.5 M, 37.4 mmol) dropwise over 10 min. The reaction was warmed to rt and stirred for 3.5 h. After cooling to 0° C., ethylene oxide (2.33 mL, 46.8 mmol) was added, the reaction was warmed to rt and stirring was continued for 19 h. The reaction was then cooled to 0° C. and quenched with sat. NH_4Cl (20 mL), followed by extraction with Et_2O (2×50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated to give 4.38 g (91%) of compound 501A as a bright orange oil. This material was used without further purification. HPLC: 94% at 2.91 min (retention time) (YMC Combiscreen ODS-A column, 4.6×50 mm, eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 4 min, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 137.13 $[M-H_2O+H]^+$.

B. (3aα,4β,7β,7aα)-4-[1,3,3a,4,7,7a-Hexahydro-4-(2-hydroxyethyl)-1,3-dioxo-7-propyl-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (501B)



A suspension of 2-(5-propyl-furan-2-yl)-ethanol (2.50 g, 16.2 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-1-yl)-1-naphthalenecarbonitrile (4.02 g, 16.2 mmol) in benzene (16 mL) was warmed to 60° C. After 3 h, the reaction was concentrated in vacuo to give a brown foam. Methanol (17 mL) was added and the mixture was sonicated to give a fine beige solid with a brown supernatant. Filtration gave 1.75 g (27%) of compound 501B as an off-white solid. This material was used without further purification. HPLC: 85% at 3.02 min & 3.14 min (atropisomers, retention time) (YMC Combiscreen ODS-A column, 4.6×50 mm, eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 4 min, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 403.31 $[M+H]^+$.

C. [3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-(2-hydroxyethyl)-1,3-dioxo-7-propyl-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (501Ci) & [3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-(2-hydroxyethyl)-1,3-dioxo-7-propyl-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (501Cii)



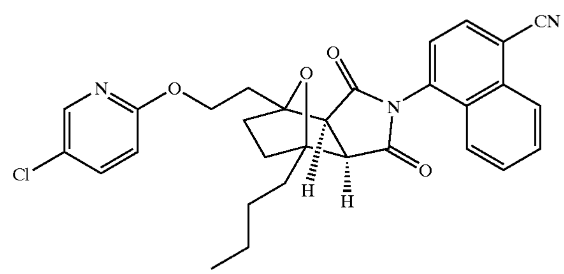
To a suspension of compound 501B (1.60 g, 3.97 mmol) in ethyl acetate (79.5 mL) was added 10% Pd/C (0.422 g, 0.397 mmol). Hydrogen gas was bubbled through the reaction for several minutes and the reaction was allowed to stir under a hydrogen atmosphere for 3 h. The reaction was filtered through Celite and the filtrate was concentrated in vacuo to give a white solid (1.74 g). The crude material was dissolved in minimum amount of methylene chloride and loaded on a 120 g silica gel ISCO cartridge. Elution with a step gradient of 0 to 100% ethyl acetate/hexane gave 1.06 g (66%) of the racemic mixture of compounds 501Ci & 501Cii as a white foam. A 500 mg portion of the racemic mixture was separated by normal phase preparative chiral HPLC (Chiralpak AD; 5×50 cm column; isocratic elution with 13% MeOH/EtOH (1:1) in heptane at 50 mL/min, monitoring at 220 nm) to give 245 mg of the faster eluting enantiomer, compound 501Ci and 245 mg of the slower eluting enantiomer, compound 501Cii, both as a white foams. Compound 501Ci: Normal phase preparative chiral HPLC: 28.0 min (retention time), >95% ee (Chiralpak AD 4.6×250 mm column, eluting with 12% MeOH/EtOH (1:1) in heptane at 1.0 mL/min). HPLC: 99% at 3.04 & 3.17 min (atropisomers, retention time) (YMC Combiscreen ODS-A column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). HRMS m/z Calc'd for $C_{24}H_{23}N_2O_4$ $[M-H]^-$: 403.1658. Found 403.1644. Compound 501Cii: Chiral HPLC: 65.7 min (retention time), >95% ee (Chiral HPLC: 65.7 min; >95% ee; Chiralpak AD 4.6×250 mm column; eluting with 12% MeOH/EtOH (1:1) in heptane at 1.0 mL/min). HPLC: 98% at 3.02 & 3.15 min (atropisomers, retention time) (YMC Combiscreen ODS-A column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). The absolute stereochemistry for compounds 501Ci & 501Cii was not established. For simplicity in nomenclature, compound 501Ci is designated herein as having an "R" configuration and compound 501Cii as having an "S" configuration. Enantiomerically pure products derived from compound 501Ci are designated herein as having a "R" configuration and enantiomerically pure products derived from compound 501Cii are designated herein as having an "S" configuration.

D. [3aR-(3aα,4β,7β,7aα)]-4-[4-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-1,3-dioxo-7-propyl-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (501D)

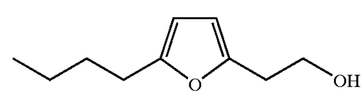
To a solution of DBAD (17.1 mg, 0.0741 mmol) in THF (0.5 mL) was added PPh_3 (19.4 mg, 0.0741 mmol). After 10 min, 5-chloro-2-pyridinol (9.6 mg, 0.074 mmol) was added. After 5 min, compound 501Ci (20.0 mg, 0.0494 mmol) was added. After 1 h, DBAD (17.1 mg, 0.0741 mmol), PPh_3 (19.4 mg, 0.0741 mmol), and 5-chloro-2-pyridinol (9.6 mg, 0.074 mmol) were added. After 3 h, the solvent was removed in vacuo to give a yellow residue. Preparative reverse phase HPLC (YMC ODS column, 20×100 mm, eluting with 40–100% aqueous methanol containing 0.1% TFA over 30 min, 25 mL/min, monitoring 220 nm) gave 9.5 mg (37%) of the trifluoracetic acid salt of compound 501D as a clear, colorless residue. HPLC: 99% at 7.88 min & 8.11 min (atropisomers, retention time) (Zorbax SB C18 4.6×75 mm, eluting with 10–90% aqueous methanol over containing 0.2% phosphoric acid over 8 min, 2.5 mL/min, monitoring at 220 nm). HRMS m/z Calc'd for $C_{29}H_{27}N_3O_4Cl$ $[M+H]^+$: 516.1690. Found 516.1676.

EXAMPLE 502

[3aR-(3aα,4β,7β,7aα)]-4-[4-Butyl-7-[2-[(5-chloro-2-pyridinyl)oxy]ethyl]octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (502D)



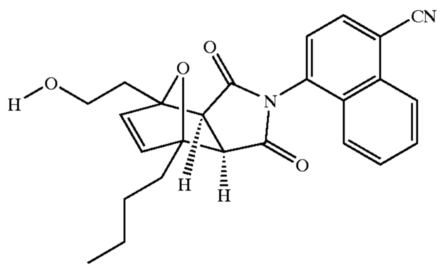
A. 2-(5-Butyl-furan-2-yl)-ethanol (502A)



To a solution of 2-butylfuran (3.00 g, 24.2 mmol) in THF (24 mL) at −78° C. was added n-BuLi (11.6 mL, 2.5 M, 29.0 mmol) dropwise over 10 min. The reaction was warmed to rt and stirred for 3.5 h. After cooling to 0° C., ethylene oxide (1.81 mL, 36.2 mmol) was added, the reaction was warmed to rt and stirring was continued for 19 h. The reaction was then cooled to 0° C. and quenched with sat. NH_4Cl (20 mL), followed by extraction with diethyl ether (2×50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give 4.07 g (100%) compound 502A as a bright orange oil. This material was used without further purification. HPLC: 96% at 3.23 min (retention time) (YMC Combiscreen ODS-A column, 4.6×50 mm, eluting with 10–90% aqueous methanol con-

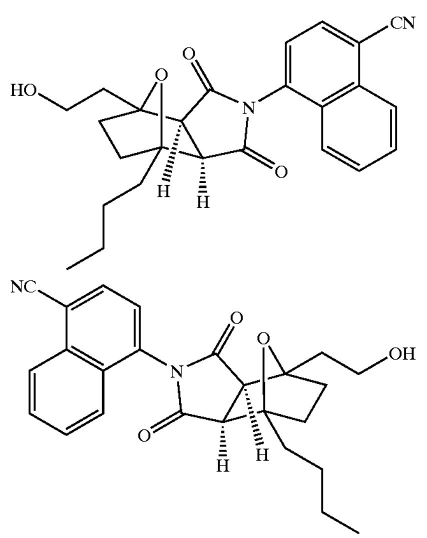
taining 0.2% phosphoric acid over 4 min, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 169.22 $[M+H]^+$.

B. (3aα,4β,7β,7aα)-4-[4-Butyl-1,3,3a,4,7,7a-hexahydro-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (502B)



A suspension of compound 502A (2.50 g, 14.9 mmol) and 4-(2,5-dihydro-2,5-dioxo-1H-1-yl)-1-naphthalenecarbonitrile (3.70 g, 14.9 mmol) in benzene (15 mL) was warmed to 60° C. After 3 h, the reaction was concentrated in vacuo to give a brown foam Methanol (17 mL) was added and the mixture was sonicated to give a fine beige solid with an orange-brown supernatant. Filtration of the precipitate gave 2.64 g (44%) of compound 502B as an off-white solid. This material was used without further purification. HPLC: 95% at 3.25 min & 3.35 min (atropisomers, retention time) (YMC Combiscreen ODS-A column, 4.6×50 mm, eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 4 min, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 417.29 $[M+H]^+$.

C. [3aR-(3aα,4β,7β,7aα)]-4-[4-Butyloctahydro-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (502Ci) & [3aS-(3aα,4β,7β,7aα)]-4-[4-Butyloctahydro-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (502Cii)



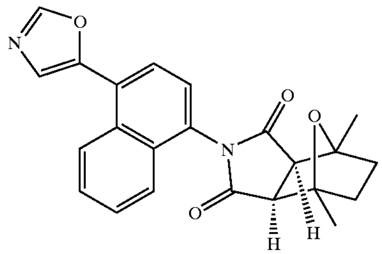
To a suspension of compound 502C (1.47 g, 3.52 mmol) in ethyl acetate (70 mL) was added 10% Pd/C (0.375 g, 0.352 mmol). Hydrogen was bubbled through the reaction for several minutes and the reaction was allowed to stir under a hydrogen atmosphere for 3 h. The reaction was filtered through Celite®, rinsing with ethyl acetate (2×70 mL). The filtrate was concentrated in vacuo to give a white foam (1.50 g). The crude material was dissolved in a minimum amount of methylene chloride and loaded on a 120 g silica gel ISCO cartridge. Elution with a step gradient of 0 to 100% ethyl acetate/hexane gave 1.05 g (74%) a racemic mixture of compounds 502Ci & 502Cii as a white foam. A 438 mg portion of racemic mixture of compounds 502Ci & 502ii was separated by normal phase preparative chiral HPLC (Chirapak AD column, 5×50 cm, isocratic elution with 12% MeOH/EtOH (1:1) in heptane at 50 mL/min, monitoring at 220 nm) to yield 178 mg of the faster eluting enantiomer, compound 502Ci as a white foam and 132 mg of the slower eluting enantiomer, compound 502Cii, as a clear, viscous oil. Compound 502Ci: Chiral HPLC: 25.5 min (retention time), >95% ee (Chiral HPLC: Chiralpak AD 4.6×250 mm column, eluting with 12% MeOH/EtOH (1:1) in heptane at 1.0 mL/min) and HPLC: 99% at 6.50 min & 6.71 min (atropisomers, retention time) (YMC Combiscreen ODS-A column, 4.6×50 mm, eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 4 min, 4 mL/min, monitoring at 220 nm) HRMS m/z Calc'd for $C_{25}H_{25}N_2O_4$ $[M-H]^-$: 417.1814. Found 417.1800. Compound 502Cii: HPLC: 55.6 min (retention time), >95% ee (Chiral HPLC: Chiralpak AD 4.6×250 mm column; eluting with 12% MeOH/EtOH (1:1) in heptane at 1.0 mL/min). HPLC: 99% at 3.26 min & 3.38 min (atropisomers, retention time) (YMC Combiscreen ODS-A column, 4.6×50 mm, eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 4 min, 4 mL/min, monitoring at 220 nm). The absolute stereochemistry for compounds 502Ci & 502Cii was not established. For simplicity in nomenclature, compound 502Ci is designated herein as having an "R" configuration and compound 502Cii as having an "S" configuration. Enantiomerically pure products derived from compound 502Ci are designated herein as having a "R" configuration and enantiomerically pure products derived from compound 502Cii are designated herein as having an "S" configuration.

D. [3aR-(3aα,4β,7β,7aα)]-4-[4-Butyl-7-[2-[(5-chloro-2-pyridinyl)oxy]ethyl]octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (502D)

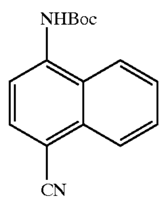
To a solution of compound 502Ci (20.0 mg, 0.0478 mmol), PPh_3 (37.6 mg, 0.143 mmol) and 5-chloro-2-pyridinol (18.6 mg, 0.143 mmol) in THF (0.5 mL) was added DBAD (33.0 mg, 0.143 mmol). The resulting solution was stirred at rt for 15.5 h. The solvent was removed in vacuo to give a yellow residue. Preparative HPLC (Shimadzu VP ODS column, 20×250 mm, eluting with 40–100% aqueous methanol containing 0.1% TFA over 30 min and 100% for 25 min, 25 mL/min, monitoring at 220 nm) gave 9.4 mg (37%) of the trifluoracetic acid salt of compound 502D as a clear, colorless residue. HPLC: 99% at 8.14 min & 8.36 min (atropisomers, retention time) (Zorbax SB C18 column, 4.6×75 mm, eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 8 min, 2.5 mL/min, monitoring at 220 nm). HRMS m/z Calc'd for $C_{30}H_{29}N_3O_4Cl$ $[M+H]^+$: 530.1847. Found 530.1855.

EXAMPLE 503

(3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-[4-(5-oxazolyl)-1-naphthalenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione (503E)

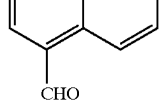


A. (4-Cyano-naphthalen-1-yl)-carbamic Acid Tert-Butyl Ester (503A)



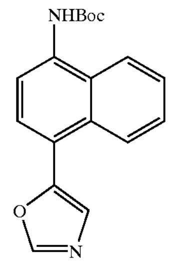
To a solution of 4-amino-1-naphthalenecarbonitrile (9.67 g, 57.5 mmol) in THF (100 mL) at rt was added, over 10 min, sodium hexamethyldisilazane (1.0 M in THF, 133 mL, 133 mmol). After stirring for 15 min, a solution of di-t-butyldicarbonate (15.1 g, 69.0 mmol) in THF (20 mL) was added. After stirring for 18 h at rt, the reaction mixture was partitioned between Et_2O (400 mL) and saturated potassium bisulfate solution (200 mL). The organic layer was washed with saturated potassium bisulfate solution (200 mL), saturated sodium bicarbonate solution (200 mL) and brine (100 mL). Drying over anhydrous magnesium sulfate, treatment with decolorizing carbon and concentration in vacuo, afforded a residue that was partially purified by flash chromatography on silica gel eluting with 20% ethyl acetate in hexane. The partially purified material was crystallized from ethyl acetate/hexane to give 5.26 g of compound 503A as a colorless crystals. The mother liquor was concentrated and crystallized from ethyl acetate/hexane to give an additional 2.8 g of compound 503A to yield a total of 8.06 g (52%) of compound 503A. ^{1}HNMR (400 MHz, DMSO-d^6): δ 9.81 (s, 1H), 8.36 (Id, 1H, J=8.5 Hz), 8.11 (m, 2H), 7.92 (d, 1H, J=8 Hz), 7.78 (m, 1H), 7.67 (m, 1H), 1.53 (s, 9H).

B. (4-Formyl-naphthalen-1-yl)-carbamic Acid Tert-Butyl Ester (503B)



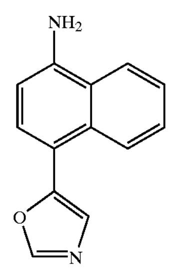
A mixture of compound 503A (4.02 g, 15.0 mmol), Raney nickel (1.5 g), sodium hypophosphite (9.00 g, 86.5 mmol), pyridine (50 mL), water (25 mL) and acetic acid (25 mL) was stirred at 45° C. for 5 h. The mixture was filtered through celite and the filter cake was rinsed with warm ethanol (100 mL). After adding water (600 mL) to the filtrate and allowing it to stand for 1 h, the resulting precipitate was filtered and rinsed with water. Drying in vacuo afforded 3.38 g of a white solid which was a 3:1 mixture of compounds 503B & 503A. The material was used in the next step without further purification. ^{1}HNMR (400 MHz, DMSO-d^6): δ 10.28 (s, 1H), 9.77 (s, 1H), 9.27 (d, 1H, J=8.5 Hz), 8.31 (m, 1H), 8.13 (m, 1H), 8.00 (d, 1H, J=8 Hz), 7.80 (m, 1H), 7.70 (m, 1H), 1.54 (s, 9H).

C. (4-Oxazol-5-yl-naphthalen-1-yl)-carbamic Acid Tert-Butyl Ester (503C)



The above mixture of compounds 503A & 503B (3.37 g, 10.0 mmol; corrected for presence of compound 503A), toluene-sulfonylisocyanide (2.15 g, 11.0 mmol) and potassium carbonate (1.66 g, 12.0 mmol) in 50 mL of methanol was refluxed for 4 h. The reaction mixture was partitioned between water (200 mL) and chloroform (200 mL). After extracting the aqueous layer with chloroform (100 mL), the combined organic layers were dried over magnesium sulfate and concentrated in vacuo. The crude residue was purified by flash chromatography on silica gel eluting with a gradient of 10–40% ethyl acetate in hexane to give 1.35 g (44%) of compound 503C as a white solid. ^{1}HNMR (400 MHz, DMSO-d^6): δ 9.45 (s, 1H), 8.57 (s, 1H), 8.20 (m, 2H), 7.76 (m, 2H), 7.64 (s, 1H), 7.62 (m, 2H), 1.51 (s, 9H).

D. 4-Oxazol-5-yl-naphthalen-1-ylamine (503D)



Compound 503C (1.34 g, mol) was dissolved in trifluoroacetic acid (10 mL) and the resulting mixture was allowed to stand for 1 h at room temperature. After removing the volatiles in vacuo, the residue was co-evaporated from ethyl acetate/heptane (2×50 mL) to remove traces of trifluoroacetic acid. After partitioning the residue between ethyl acetate (100 mL) and 1N NaOH (75 mL), the organic layer was washed with brine (50 mL), dried over magnesium sulfate and concentrated in vacuo to afford 900 mg (99%) of compound 503D as a yellow crystalline solid. HPLC conditions: 95% at 0.92 min (retention time) (Phenomenex 5

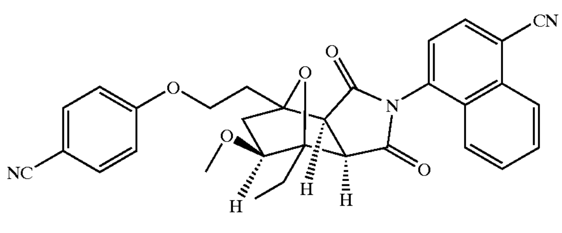
micron ODS column, 4.6×30 mm, 10%–90% aqueous methanol over 2 min gradient with 0.1% TFA, monitoring at 254 nm.). MS (ES): m/z 211.22 [M+H]$^+$.

E. (3aα,4β,7β,7aα)-Hexahydro-4,7-dimethyl-2-[4-(5-oxazolyl)-1-naphthalenyl]-4,7-epoxy-1H-isoindole-1,3(2H)-dione (503E)

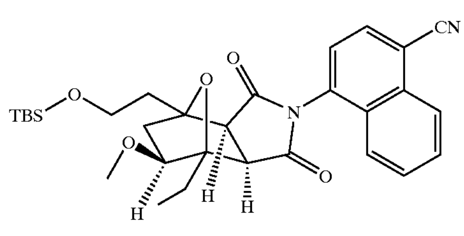
A mixture of compound 503D (42 mg, 0.020 mmol) and 20A (78 mg, 0.40 mmol) in acetic acid (1.0 mL) was refluxed for 18 h. The reaction mixture was cooled to rt, concentrated in vacuo and the residue was partitioned between ethyl acetate (30 mL) and saturated sodium bicarbonate solution (30 mL). The organic layer was isolated, dried over magnesium sulfate and concentrated in vacuo. Purification by flash chromatography on a 2.5×15 cm silica gel column, using a gradient of 40–60% ethyl acetate in hexane gave 28 mg (37%) of compound 503E as a white powder. HPLC: 99% at 1.46 min & 1.36 min (atropisomers, retention time) (Phenomenex 5 micron ODS 4.6×30 mm, 10%–90% aqueous methanol over 2 min gradient with 0.1% TFA, monitoring at 254 nm.). MS (ES): m/z 389.10 [M+H]$^+$.

EXAMPLE 504

[3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(4-Cyanophenoxy)ethyl]-4-ethyloctahydro-5-methoxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (504C)

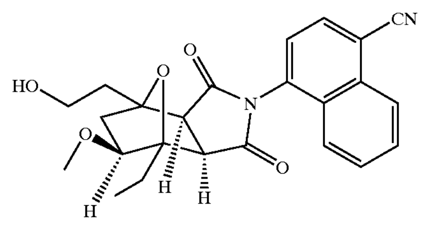


A. [3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-4-ethyloctahydro-5-methoxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (504A)



To a solution of compound 499ii (0.235 g, 0.451 mmol) in CH_3CN (6 mL) was added silver oxide (0.523 g, 2.26 mmol) and iodomethane (0.56 mL, 9.0 mmol, stirred over K_2CO_3 before addition). The resulting suspension was placed in a preheated oil bath (80° C.). After 24 h, the reaction was cooled to rt, diluted with CH_3CN (20 mL), filtered through a plug of Celite, and concentrated in vacuo to give a brown gum. Purification by flash chromatography on silica gel eluting with 30% ethyl acetate/hexanes gave 0.156 g (65%) of compound 504A as a white solid. HPLC: 95% at 4.17 mm & 4.25 min (atropisomers, retention time) (YMC CombiSreen ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 4 min, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 521.25 [M+H]$^+$.

B. (3aα,4β,5β,7β,7aα)-4-[4-Ethyloctahydro-7-(2-hydroxyethyl)-5-methoxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (504B)



To a solution of compound 504A (0.156 g, 0.292 mmol) in ethanol (6 mL) was added 1N HCl (0.44 mL, 0.44 mmol). After 20 min, the reaction was cooled to 0° C. and quenched with sat. aq. $NaHCO_3$ (2 mL) to give a white suspension. Added H_2O until the solid dissolved. The mixture was extracted with ethyl acetate (3×30 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to give a white solid. Purification by flash chromatography on silica gel eluting with 5% $MeOH/CH_2Cl_2$ gave 120 mg (99%) of compound 504A as a white solid. HPLC: 98% at 5.17 and 5.44 min (atropisomers, retention time) (YMC CombiSreen ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 8 min, 2.5 mL/min, monitoring at 220 nm). HRMS m/z Calc'd for $C_{24}H_{24}N_2O_5$ [M−H]$^+$: 419.1607. Found 419.1611.

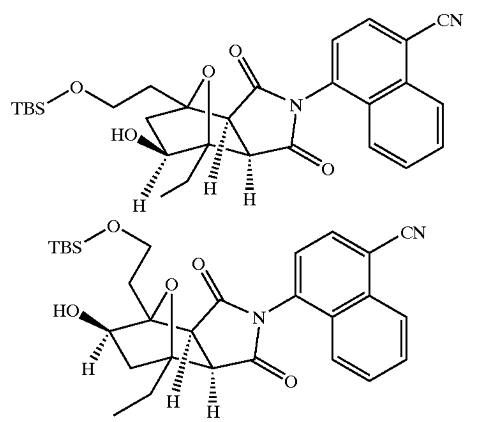
C. [3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(4-Cyanophenoxy)ethyl]-4-ethyloctahydro-5-methoxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (504C)

To a solution of compound 504B (20 mg, 0.048 mmol) in anhydrous THF (0.5 mL) was added PPh_3 (37.0 mg, 0.143 mmol), para-cyanophenol (17.0 mg, 0.143 mmol) and DBAD (32.0 mg, 0.143 mmol). After 30 min, the solution was concentrated to give a brown gum. Purification by

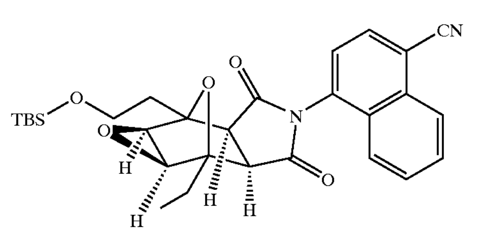
reverse phase preparative HPLC (YMC S5 ODS column, 20×250 mm, eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 35 min, 20 mL/min, monitoring at 220 nm) gave 16 mg (64%) of compound 504C as a clear, colorless oil. HPLC 98% at 6.84 and 7.10 min (atropisomers, retention time) (Zorbax SB C18 column, 4.6×75 mm, eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 8 min, 2.5 mL/min, monitoring at 220 nm). HRMS m/z Calc'd for $C_{31}H_{27}N_3O_5$ $[M+NH_4]^+$: 539.2295. Found 539.2302.

EXAMPLE 505

(3aα,4β,5β,7β,7aα)-4-[7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-4-ethyloctahydro-5-hydroxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (505Bi) & (3aα,4β,5β,7β,7aα)-4-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-7-ethyloctahydro-5-hydroxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (505Bii)



A. (1aα,2β,2aα,5aα,6β,7aα)-4-[2-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-6-ethyloctahydro-3,5-dioxo-2,6-epoxy-4H-oxireno[f]isoindol-4-yl]-1-naphthalenecarbonitrile (505A)



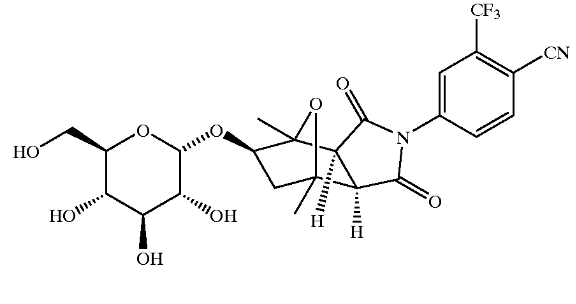
To a solution of compound 434B (1.01 g, 2.01 mmol) in methylene chloride (20 mL) was added 60% m-CPBA (0.863 g, 3.00 mmol). After 48 h, the reaction was diluted with methylene chloride (50 mL) and washed with sat. Na_2SO_3 (20 mL) and sat. $NaHCO_3$ (20 mL). The combined aqueous layers were extracted with CH_2Cl_2 (20 mL). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to give 1.01 g (97%) of compound 505A as a yellow solid. This material used without further purification. HPLC: 95% at 4.22 min (retention time) (Phenominex Luna C18 column, 4.6×50 mm, eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 4 min, 4 mL/min, monitoring at 220 nm). HRMS m/z Calc'd for $C_{29}H_{34}N_2O_5Si$ $[M-H]^+$: 517.2159. Found 517.2163.

B. (3aα,4β,5β,7β,7aα)-4-[7-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-4-ethyloctahydro-5-hydroxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (505Bi) & (3aα,4β,5β,7β,7aα)-4-[4-[2-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]ethyl]-7-ethyloctahydro-5-hydroxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (505Bii)

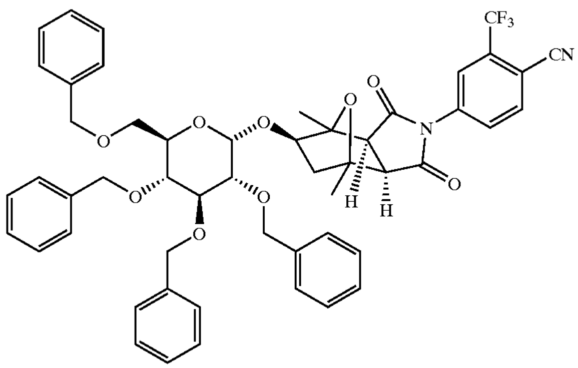
To a red solution of titanocene dichloride (0.500 g, 2.00 mmol) in anhydrous THF (4.0 mL) was added zinc dust (0.392 g, 6.00 mmol). The resulting suspension was vigorously stirred for 1 h under an argon atmosphere to give a green suspension. Excess zinc was removed by filtration through a 0.45 μm microfilter to give a green solution of dicyclopentadienyl titanium (m) chloride. To a solution of compound 505A (0.207 g, 0.399 mmol) and 1,4-cyclohexadiene (0.380 mL, 4.02 mmol) in anhydrous THF (1 mL) was added dropwise a 0.5 M solution of the above described dicyclopentadienyl titanium (III) chloride (0.9 mL, 0.45 mmol). After 1 h, an additional aliquot of the 0.5 M solution of dicyclopentadienyl titanium (III) chloride (0.9 mL, 0.45 mmol) was added and stirring was continued for 1 h. The reaction was then quenched with water (2 mL) and diluted with ethyl acetate (10 mL). The layers were separated and the organic layer was washed with brine (5 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to give a yellow gum. The crude material was dissolved in a minimum amount of methylene chloride and loaded on a 35 g silica gel ISCO column. Gradient elution with 0–80% ethyl acetate in hexane gave 0.043 g (21%) of compound 505Bi as a white solid and 0.023 g (11%) of compound 505Bii as a white solid. Compound 505Bi: HPLC: 3.92 min (retention time) (YMC CombiSreen ODS-A column, 4.6×50 mm, eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 4 min, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 521.36 $[M+H]^+$. Compound 505Bii: HPLC: 91% at 3.97 min (retention time) (YMC CombiScreen ODS-A column, 4.6×50 mm, eluting with 10–90% aqueous methanol containing 0.2% phosphoric acid over 4 min, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 521.34 $[M+H]^+$.

EXAMPLE 506

4-[[3aS-(3aβ,4β,5β,7β,7aα)]-5-(α-D-Glucopyranosyloxy)octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (506B)



A. [3aS-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-4,7-dimethyl-1,3-dioxo-5-[[2,3,4,6-tetrakis-O-(phenylmethyl)-α-D-glucopyranosyl]oxy]-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (506A)



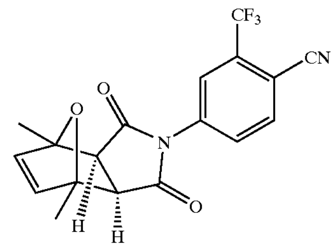
2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl bromide was made according to the procedure by Spohr et al. *Can. J. Chem.* 71, 1928–42 (1993). Oxalyl bromide (0.48 mL, 0.95 mmol, 2 M in CH_2Cl_2) was added dropwise to a solution of 2,3,4,6-tetra-O-benzyl-D-glucopyranose (412 mg, 0.763 mmol) in CH_2Cl_2 (5 mL) and DMF (0.28 mL) at rt under Ar. The reaction mixture was stirred for 20 min, poured onto a mixture of ice and H_2O (1:1, 30 mL) and diluted with CH_2Cl_2 (30 mL). The layers were separated and the organic layer was washed with cold H_2O (2×30 mL) and brine (1×30 mL) and dried over $MgSO_4$. Concentration in vacuo gave the desired bromide as a brown oil. This oil was taken up in CH_2Cl_2 (2 mL) and DMF (1 mL). Compound 471Dii (100 mg, 0.763 mmol), tetrabutylammonium bromide (111 mg, 0.526 mmol) and 4 Å sieves (600 mg) were added to this solution and the reaction was stirred under Ar for 4 d. The reaction was quenched with MeOH (2 mL), stirred for 0.5 h, diluted with CH_2Cl_2 (10 mL) and then filtered through a medium porosity fritted funnel, rinsing with CH_2Cl_2 (5 mL). The solvent was removed in vacuo and the resulting residue was dissolved in CH_2Cl_2 (25 mL). The organic solution was washed with sat. aq. $NaHCO_3$ (1×20 mL) and H_2O (1×20 mL) and dried over $MgSO_4$. Purification by flash chromatography on SiO_2 eluting with 50% EtOAc/hexanes gave 79 mg (33%) of 506A as a white solid. HPLC: 99% at 4.56 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 902 $[M+H]^+$.

B. 4-[[3aS-(3aα,4β,5β,7β,7aα)]-5-(α-D-Glucopyranosyloxy)octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (506B)

Palladium hydroxide (62 mg, 20 wt. % Pd (dry basis) on carbon, wet) was added to a solution of 506A (65 mg, 0.07 mmol) in EtOAc (2 mL) and the mixture was stirred under a hydrogen atmosphere introduced via a balloon. After 5 h, the reaction was complete as was evident by HPLC, so the mixture was filtered through a medium porosity fritted funnel rinsing with MeOH (2 mL) and concentrated in vacuo. The resulting residue was dissolved in MeOH (2 mL) and filtered through a Gelman Acrodisc CR 13 mm syringe filter with a 0.45 μM PTFE membrane. Concentration yielded 38 mg of 506B as a white solid. HPLC: 90% at 2.16 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 543.20 $[M+H]^+$. A 10 mg portion was recrystallized from MeOH:H_2O to give crystals suitable for X-ray crystal diffraction studies to elucidate the exact stereochemistry of compound 506B as referenced to the known fixed stereochemistry of the D-glucoside appendage.

EXAMPLE 507

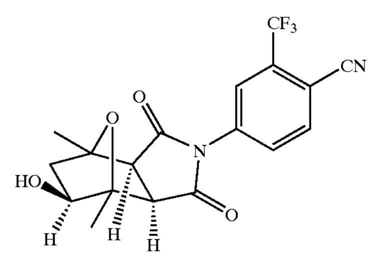
(3aα,4β,7β,7aα)-4-(1,3,3a,4,7,7a-Hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (507)



To 25 g (94.2 mmol) of compound 471A was added neat 2,5-dimethylfuran (30 mL, 280 mmol), and the resulting slurry was heated to 60° C. for 1 to 3 h with mechanical agitation. The resulting slurry was cooled to 0–5° C., and diluted with cold toluene (25 mL, 0–10° C.). The cold slurry was filtered under vacuum. The flask and filter cake were washed with cold toluene (2×25 mL), and the cake was deliquored with house vacuum. The precipitate was dried in vacuo to yield 31.3 g (91.6%) of a compound 507 as a tan solid. HPLC: 99.6%, 19.43 min (retention time) (Column: Zorbax™ SB-C18, 4.6×15 cm; Mobile Phase: 40% CH_3CN/60% H_2O w/0.1% v/v TFA, isocratic; Flow Rate: 1 mL/min; Detection: λ_{max} 210 nm; Temperature: 30° C.; Injection Volume: 5 μL).

EXAMPLE 508

(3aα,4β,5β,7β,7aα)-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (508)

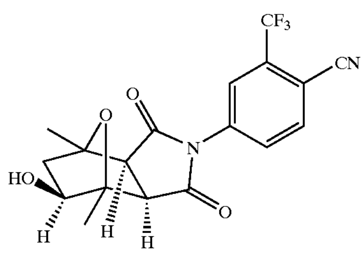


To THF (275 mL) that had been cooled to −3° C. was added compound 507 (55.0 g, 152 mmol), which resulted in a slurry. To the slurry was added borane methylsulfide (14.4 mL, 152 mmol), at a rate such that the temperature did not exceed 0° C. The reaction mixture was slowly warmed to 20° C. over 2.5 h. The temperature of the reaction mixture was then returned to 0° C., where upon phosphate buffer (1056 mL, pH 7) was carefully added at a rate to control the hydrogen gas evolution and maintain a temperature ≦20° C.

The resulting suspension was dissolved by adding ethanol (528 mL, 190 proof). At 15° C., hydrogen peroxide (55 mL, 30 wt %) was added at a rate to maintain the temperature ≦20° C. The homogeneous solution was left stirring for 12 h at 20° C. and pH 7.8, whereby crystallization occurred. The resulting slurry was collected by filtration and washed with water (4×100 mL) and methyl-tert-butyl ether (2×100 mL). Drying in vacuo afforded 37.3 g (64.6%) of compound 508. The aqueous mother liquor was extracted with ethyl acetate (3×500 mL). The combined rich organics were washed with 10 wt % aqueous sodium sulfite (1×100 mL), and 25 wt % aqueous sodium chloride (1×100 mL). The organics were dried over sodium sulfate, filtered, and concentrated to recover 8.9 g (15.4%) of compound 508, and a third 11.5 g (20%) fraction of compound 508 was recovered from the methyl-tert-butyl ether cake wash. The above three solid samples of crude material were separately recrystallized from 190 proof ethanol (1 g/10 mL) to afford a total of 35.6 g (61.7%) of compound 508 having a purity level of 98.7% as determined by HPLC analysis (conditions as below). A second crop of compound 508 was isolated from the mother liquor to afford 9.3 g (16.1%) of solid having a purity level of 98.4% as determined by HPLC analysis. The remaining mother liquor was purified by silica gel chromatography using 200 g of SiO_2 and eluting with 4 L of 50V % ethyl acetate and 50 V % heptane to yield 5.6 g (9.7%) of compound 508, having a purity level of 94.0% as determined by HPLC analysis. HPLC conditions: 9.74 minute retention time on a YMC S5 ODS-AQ column (4.6×150 mm) using a gradient elution from 100% solvent A to 100% solvent B over 15 minutes at 1.0 mL/min. Solvent A=95 V % water (0.01 M NH_4OAc); 5 V % acetonitrile. Solvent B=5 V % water (0.01 M NH_4OAc); 95 V % acetonitrile. Duel wavelength detector set at 210 and 245 nm. MS (ES): m/z 381.11 $[M+H]^+$.

EXAMPLE 509

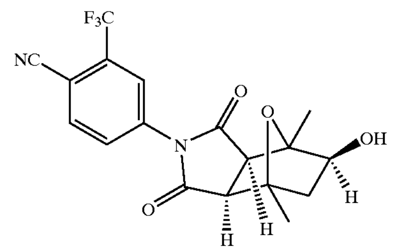
(3aα,4β,5β,7β,7aα)-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (509)

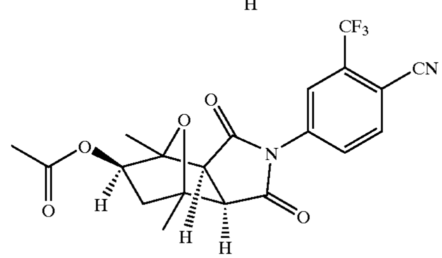


Compound 471A (0.37 g, 1.4 mmol) and 2,5-dimethylfuran (0.73 mL, 6.9 mmol) were combined to form a slurry which was heated to 60° C. for 1 h. The reaction mixture was cooled to −10° C. and THF (1.0 mL) was added followed by the addition of borane tetrahydrofuran (2.1 mL, 1 M). The reaction mixture was stirred for 30 minutes at 0° C. and 30 minutes at +10° C. To the reaction mixture was added acetone (3.0 mL), and the resulting mixture was warmed to 20° C. and maintained at 20° C. for 1 h. To this solution was added sodium bicarbonate (1.5 mL, pH 9, 8 wt %) and the mixture was then cooled to 5° C. before adding hydrogen peroxide (0.3 mL, 30 wt %). Addition of hydrogen peroxide was exothermic bringing the temperature to 20° C. A solution of sodium sulfite (4.0 mL, 10 wt %) was added at 20° C. resulting in an exotherm to 30° C. The biphasic mixture was allowed to stand at 25° C. for 12 h. After the phases were separated, the aqueous waste was back extracted twice with ethyl acetate (5 mL) and the combined organic layers were washed with water (2 mL) followed by sodium chloride (2 mL, 25 wt %). The organic layers were concentrated in vacuo to yield a yellow oil which rapidly crystallized. To the crude product was added 190 proof ethanol (5.0 mL) and the mixture was heated to 60° C. to afford complete dissolution. Cooling to 20° C. for 17 h resulted in crystallization. The crystal slurry was collected by filtration, washed with heptane (5 mL), and dried at 60° C. under vacuum (30 in/Hg) to afford 0.23 g (44%) of compound 509 having 93.1 HPLC Area %. HPLC conditions: 9.74 minute retention time on a YMC S5 ODS-AQ column (4.6×150 mm) using a gradient elution from 100% solvent A to 100% solvent B over 15 minutes at 1.0 mL/min. Solvent A=95 V % water (0.01 M NH_4OAc); 5 V % acetonitrile. Solvent B=5 V % water (0.01 M NH_4OAc); 95 V % acetonitrile. Detector set at 245 nm. MS (ES): m/z 381.11 $[M+H]^+$.

EXAMPLE 510

[3aR-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (510i) & [3aS-(3aα,4β5β,7β,7aα)-4-[5-(Acetyloxy)octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (510ii)





Compound 509 (4 mg), vinyl acetate (0.1 mL) and toluene (2 mL) were combined and 20 mg of each of the enzymes shown in Table 12 were added. The mixture was stirred with a magnetic stirring bar at rt in a 16×100 mm capped tube for the time period listed in Table 12. The enantioselective acetylation of the racemic mixture resulted in the formation of compound 510i and the acetylated compound 510ii. The enantiomeric purity of compound 510i was determined by chiral HPLC (method below) and the results for each enzyme are as shown in Table 12. The resulting information was used to prepare a large scale batch of compounds 510i & 510ii as described below.

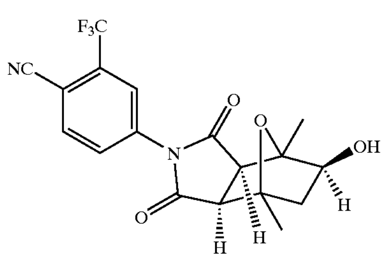
TABLE 12

Enzyme	Supplier	Source	Time H	Comp. 510i mg/mL	Comp. 510i % yield	Comp. 510i % ee	Comp. 510ii mg/mL
AK-20	Amano	*Pseudomonas fluorescens*	15	0.74	39	100	1.02
AP-12	Amano	*Aspergillus niger*	144	1.10	58	55.4	0.74
PS-30	Amano	*Pseudomonas cepacia*	15	0.46	24	100	1.24
Acylase 30000	Amano	*Aspergillus*	15	0.51	27	12.2	1.26
Chirazyme L3	Boehringer	*Candida rugosa*	144	0.81	42	87.2	1.04
Lipase type VII	Sigma	*Candida rugosa*	144	0.74	39	100	1.06

To a 500 mL jacketed flask were added Amano lipase AK20 from *Pseudomonas fluorescens* (25 g), compound 509 (25 g), methyl-isobutyl-ketone (475 mL) and vinyl acetate (25 mL). The flask was maintained at 25° C. with a circulating water bath and stirred with a magnetic stir bar. The incubation was continued for 42 h, at which point the enantiomeric excess of compound 510i reached 100%. The solution was filtered through Whatman 4 filter paper to remove enzyme and the filter cake was washed with 50 mL methyl isobutyl ketone. The filtrate was concentrated in vacuo and the resulting residue was dissolved in EtOAc (50 mL) followed by the addition of heptane (50 mL). This solution was loaded onto a Phenomenex cartridge column (silica 800 g) in a Biotage 75L system and the column was eluted with 75% EtOAc/heptane at flow rate 110 mL/min. Fractions were collected (500 mL) which contained compound 510ii and then the eluting solvent was changed to 100% EtOAc to elute off compound 510i. The desired fractions were pooled and the solvent was removed in vacuo to yield 11.0 g of compound 510i, (44%, 100% ee) and 12.10 g of compound 510ii (44%). Compound 510i was recrystallized from 95% EtOH (5 mL/g) in two crops to afford 9.61 g (38%) of compound 510i as a white crystalline solid. Compounds 510i: Chiral HPLC: 10.02 min (retention time) (CHIRALPAK AD 4.6×250 mm column; isocratic elution with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min). HPLC: 99% at 2.45 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 381.11 $[M+H]^+$.

EXAMPLE 511

[3aR-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (511i) & Butanedioic Acid, mono[3aS-(3aα,4β,5β,7β,7aα)]-[2-[4-cyano-3-(trifluoromethyl)phenyl]octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-1H-isoindol-5-yl] ester—(511ii)



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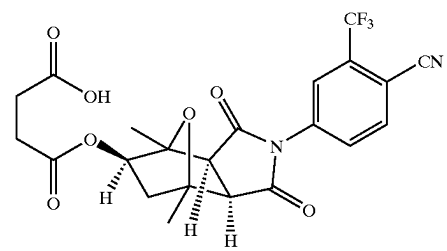


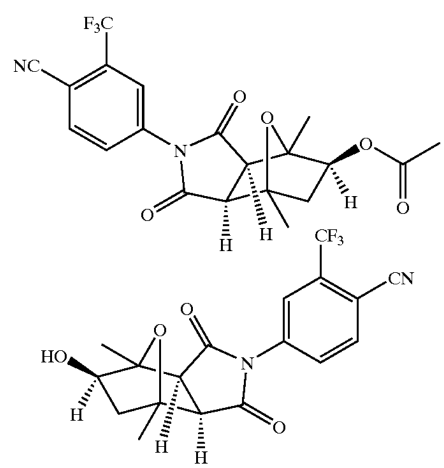
A mixture of the racemic compound 509 (10 mg), succinic anhydride (100 mg) and lipase AK-20 Amano (50 mg) in toluene or MTBE (5 mL) was stirred at rt for 20 hours. After 16 and 20 h, samples (0.1 mL) were taken out from each reaction mixture, evaporated, redissolved in acetonitrile (1 mL) and analyzed by reversed phase HPLC (YMC Pro-pack ODS-A, 3μ, 15×0.6 cm, acetonitrile:water 20:80 to 90:10 in 12 min) to determine the area ratio of products compound 511i (RT=8.8 min) and compound 511ii (RT=9.9 min). A second sample (0.1 mL) of each reaction mixture was removed, evaporated and redissolved in 1 mL isopropyl alcohol-heptane (1:1) and analyzed by Chiral HPLC (Chiralpak AD, 25×0.46 cm, 20° C., heptane:ethanol 85:15, 0.5 mL/min, UV 210 nm) to determine the % ee of compound 511i (RT=32.2 min) and compound 471Dii (RT=34.8 min). After 20 h, the reaction mixtures were filtered off to separate the insoluble components (enzyme, etc.). The filtrates were washed with 5% aqueous $NaHCO_3$ (3×1 volume) and water (3×1 volume), evaporated in vacuo and analyzed by HPLC as described above. The results showed an average yield of 48% (theoretical max yield is 50%) and 100% ee for compound 511i. Complete separation of compound 511ii was achieved via the above described $NaHCO_3$ extraction. Table 13 gives the details of each reaction as determined by the methods described above. Compound 511i: Chiral HPLC: 10.02 min; CHIRALPAK AD 4.6×250 mm column; isocratic elution with 20% MeOH/EtOH (1:1) in heptane at 1 mL/min, 100% ee. HPLC: 99% at 2.45 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 381.11 $[M+H]^+$.

TABLE 13

Solvent	Solvent Vol., ml	Comp. 509 mg	Lipase AK-20 mg	Succinic Anhydride mg	Time Hr	Comp. 511i 8.8 min Area Ratio	Comp. 511ii 9.9 min Area Ratio	Comp 511i 32.2 min %	Comp 471Dii 34.8 min %	Comp. 511i ee %
Toluene	5	10	50	100	16	53%	47%	93.1%	6.9%	86.2%
					20	54%	46%	96.0%	4.0%	92.0%
Toluene Wash NaHCO3						100%	0%	96.1%	3.9%	92.2%
MTBE	5	10	50	100	16	49%	51%	100.0%	0.0%	100.0%
					20	50%	50%	100.0%	0.0%	100.0%
MTBE Wash NaHCO3						100%	0%	100.0%	0.0%	100.0%

EXAMPLE 512

[3aR-(3aα,4β,5β,7β,7aα)-4-[5-(Acetyloxy) octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-(trifluoromethyl)benzonitrile (512i) & [3 aS-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (512ii)



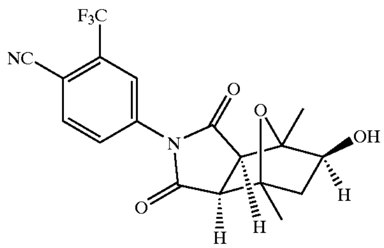
A series of 50 mL flasks were arranged and enzymes (see Table 14 for enzyme type and amounts) were weighed into each followed by the addition of phosphate buffer (BF45, 5 mL, 100 mM, pH 7). A solution of compound 473 (5 mg) in DMSO (50 μL) was added to each flask. The flasks were shaken at 200 rpm at 28° C. for 24 hours. After 24 hours, the reaction mixtures were extracted with EtOAc (10 mL). A portion of the EtOAc extract (1 mL) was evaporated, redissolved in acetonitrile (1 mL) and analyzed by reversed phase HPLC (C-18, acetonitrile:water 20:80 to 90:10 in 12 min) to determine the area ratio of compound 512i (RT=11.0 min) and compound 512ii, (RT=8.9 min). Another portion of EtOAc extract (4 mL) was evaporated, redissolved in 1 mL isopropyl alcohol-heptane (1:1) and analyzed by chiral HPLC (Chiralpak AD, heptane:ethanol 85:15, 0.5 mL/min) to determine the % ee of the compound 512ii (RT=34.8 min) and compound 471Di (RT=32.2 min) in this system. Table 14 gives details for an array of different enzymes examined and the resulting yields and % ee for the desired products.

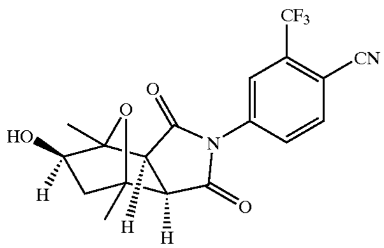
TABLE 14

Enzyme	Supplier Source	Enz Amt mg	Area Ratio by HPLC Comp. 512ii	Area Ratio by HPLC Comp. 512i	Exo-Alcohol Comp 471Di	Exo-Alcohol Comp. 511ii	% ee of Comp 512ii
Lipase AP-12	Amano *Aspergillus niger*	5	18%	82%	18.8%	81.2%	62.5%
Lipase AP-12	Amano *Aspergillus niger*	25	50%	50%	41.0%	59.0%	17.9%
Lipase PS	Amano *Pseudomonas cepacia*	5	20%	80%	31.3%	68.7%	37.4%
Lipase PS	Amano *Pseudomonas cepacia*	25	46%	54%	40.2%	59.8%	19.6%
Acylase 150000	Amano *Aspergillus* sp	5	30%	70%	52.0%	48.0%	−3.9%
Acylase 150001	Amano *Aspergillus* sp	25	71%	29%	50.4%	49.6%	−0.8%
Newlase F	Amano *Rhizopus niveus*	50	3%	97%	31.3%	68.7%	37.5%
Newlase F	Amano *Rhizopus niveus*	100	3%	97%	25.7%	74.3%	48.5%
Acylase I	Sigma *Apergillus melleus*	5	10%	90%	9.7%	90.3%	80.6%
Acylase I	Sigma *Apergillus melleus*	25	38%	62%	10.9%	89.1%	78.3%
Esterase	Sigma Porcine liver	5	78%	24%	36.0%	64.0%	27.9%

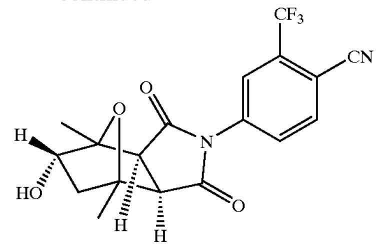
EXAMPLE 513

[3aR-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (513i) & [3aS-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile & (513ii) & (3aα,4β,5α,7β,7aα)-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (513iii)





-continued



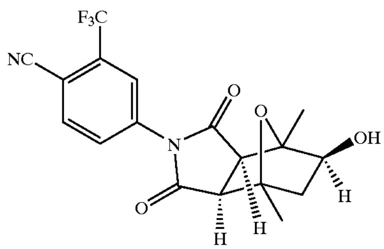
A series of microbial biotransformation reactions were set up to generate compounds 513i, 513ii & 513iii. The details of the reactions for several microorganisms are shown in Table 15 and a general procedure is described below. One thawed vial of the microbe (1 m/L culture) was inoculated into sterile soybean-glucose media (10 mL) in a 50 mL flask. The microbes were grown by shaking at 200 rpm at 28° C. for 40 h. A solution of compound 472 (10 mg in 100 μL DMSO) was added to each flask and the flasks were shaken at 200 rpm at 28° C. At 24 and 48 h, 5 mL of the reaction mixtures were extracted by EtOAc (10 mL). A portion of EtOAc extract (1 mL) was evaporated, redissolved in acetonitrile (1 mL) and analyzed by reversed phase HPLC (C-18, acetonitrile: water 20:80 to 90:10 in 12 min) to determine the area ratio of compound 472 (RT=11.2 min) and the product compounds 513i (RT=8.9 min), 513ii (RT=8.9 min) & 513iii (RT=9.6 min). A second portion of the EtOAc extract (4 mL) was evaporated, redissolved in isopropyl alcohol-heptane (1:1, 1 mL) and analyzed by chiral HPLC (Chiralpak AD, Heptane:Ethanol 85:15, 0.5 mL/min) to determine the % ee of compounds 513i (RT=32.2 min) compound 513ii (RT=34.8 min) in this system.

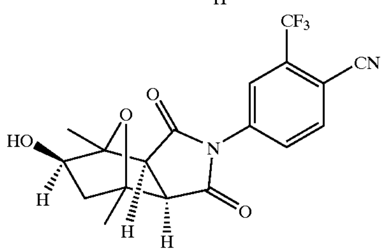
TABLE 15

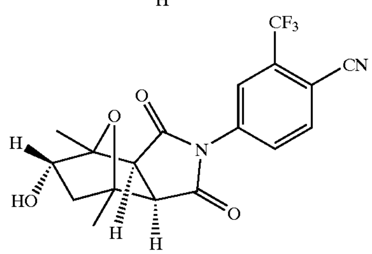
		Time	Analysis by Reversed Phase HPLC (Area Ratio)			Analysis for % ee by HPLC		% ee of
Microorganism	ID	Hrs	Comp 513i	Comp 513ii	Comp 472	Comp 513i	Comp 513ii	Comp 513i
Streptomyces sp	SC1754	24	7%	0%	93%	96.9%	3.1%	93.9%
		48	12%	0%	88%	96.7%	3..3%	93.3%
Streptomyces sp	SC3740	24	1%	0%	99%	88.0%	12.0%	76.0%
		48	2%	0%	98%	85.8%	14.2%	71.7%
Nocardia interforma	ATCC 21072	24	5%	0%	95%	93.2%	6.8%	86.3%
		48	8%	0%	92%	92.6%	7.4%	85.1%
Streptomyces antibioticus	ATCC 14890	24	11%	1%	88%	95.7%	4.3%	91.4%
		48	48%	13%	39%	94.1%	5.9%	88.2%
Streptomyces mediocidicus	TCC 13278	24	1%	0%	99%	82.0%	18.0%	64.1%
		48	7%	0%	93%	79.4%	20.6%	58.8%
Streptomyces griseus	NRRL B8090	24	23%	0%	77%	85.0%	15.0%	70.0%
		48	28%	0%	72%	85.9%	14.1%	71.8%
Amycolatopsis orientalis	ATCC 43490	24	12%	1%	86%	81.3%	18.7%	62.5%
		48	25%	4%	71%	77.4%	22.6%	54.9%

EXAMPLE 514

[3aR-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (514i) & [3aS-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile & (514ii) & (3aα,4β,5α,7β,7aα)-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-2-(trifluoromethyl)benzonitrile (514iii)







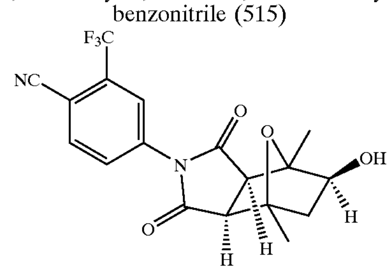
A series of microbial biotransformation reactions were set up to generate compounds 5141,514ii & 514iii. The details of the reactions for several microorganisms are shown in Table 16 and a general procedure is described below. A 1 mL culture of *Streptomyces griseus* SC13971 from a frozen vial was used to inoculate 100 mL of medium (0.5% toasted nutrisoy, 2% glucose, 0.5% yeast extract, 0.5% K_2HPO_4, 0.5% NaCl, adjusted to pH 7 with 1N HCl (R. V. Smith and J. P. Rosazza, Arch. Biochem. Biophys., 161, 551–558 (1974)) in a 500 mL Erlenmeyer flask and the flask was incubated at 28° C. at 200 rpm for 3 days. 10 mL of this culture was used to inoculate 100 mL of medium (as above) in a 500 mL Erlenmeyer flask and the flask was incubated at 28° C. at 200 rpm for 1 day. For the filamentous fungi *Mucor rouxii* and *Cunninghamella echinulata,* 1 mL of spore suspension, prepared by washing a slant with 10 mL water, was used to inoculate 100 mL of medium (as above) in a 500 mL Erlenmeyer flask and the flask was incubated at 28° C. at 200 rpm for 1 day. Compound 472 (30 mg in 1 mL methanol) was added to each culture and the incubations were continued for 6 to 10 days. Samples of 10 mL of the culture broth in each flask were removed and extracted with ethyl acetate (20 mL). Samples of 10 mL of the organic layers were each individually evaporated to dryness at 40° C. under a nitrogen stream. The residues were dissolved in 1.2 mL isopropanol and analyzed by reversed phase HPLC (YMC Pak ODS 150×6 mm, 3μ C-18, acetonitrile: water 20:80 to 90:10 in 12 min, 1 mL/min, 40° C.) to determine the concentration of compound 472 (RT=11.2 min) and product compounds 514i (RT=8.9 min), 514ii (RT=8.9 min) & 514iii (RT=9.6 min). The same samples were analyzed by chiral HPLC (Chiralpak AD, heptane:ethanol 85:15, 0.5 mL/min) to determine the % ee of compound 514i (RT=32.2 min) compound 514ii (RT=34.8 min) in this system.

TABLE 16

strain	SC	ATCC	time days	Comp. 472 mg/ml	Comp. 514i mg/ml	Comp 514i ee %	Comp 514iii mg/ml
1. *Mucor rouxii*	13920	24905	3	0.30	0.01	100.00	0.000
			6	0.27	0.01	100.00	0.000
2. *Streptomyces griseus*	13971	13273	3	0.29	0.01	100.00	0.000
			6	0.30	0.01	100.00	0.000
3. *Cunninghamella echinulata*	16027	9244	3	0.34	0.02	100.00	0.002
			6	0.06	0.02	100.00	0.001
			10	0.16	0.02	100.00	0.001

EXAMPLE 515

[3aR-(3aα,4β,5β,7β,7aα)]-4-(Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-fluoromethyl) benzonitrile (515)



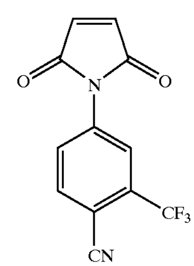
Microbial transformation of compound 472 to compound 515 was conducted on a 3 L scale in a 5 L fermentor, using *Cunninghamella echinulata* SC 16027 (ATCC 9244) and a medium consisting of the following: 0.5% toasted nutrisoy, 2% glucose, 0.5% yeast extract, 0.5% K_2HPO_4, 0.5% NaCl, adjusted to pH 7 with 1N HCl (R. V. Smith and J. P. Rosazza, Arch. Biochem. Biophys., 161, 551–558 (1974)). The fermentor was batched with 0.05% SAG antifoam before sterilization. Spore inoculum was prepared by washing the spores from a 10 day slant culture of *Cunninghamella echinulata* SC 16027 (ATCC 9244) with 0.9% saline/0.1% Tween 80. The inoculum stage was prepared by adding 1 mL of spore inoculum into 100 mL medium in a 500 mL flask, then the cultures were grown at 28° C. at 200 rpm for 1 day. 10% inoculum from the flask was blended in a sterile Waring blender and used to inoculate the sterile fermentor, containing 3 L of sterile media. The fermentor was run at 28° C. at 600 rpm and 1 vvm aeration. A sterile solution of three antibiotics was added to the fermentor after inoculation; 12 mg of tetracycline chloride, 12 mg of kanamycin sulfate, and 60 mg of cephalexin hydrate in 10 mL of deionized water. After 22 hours of growth in the fermentor, a sterile substrate solution was added containing 0.75 g of compound 472 dissolved in 30 mL of methanol, this step was repeated two hours later for a total of 0.5 g/L of compound 472 in the bioconversion. The fermentation conditions were maintained at 28° C. at 600 rpm and 1 vvm aeration. pH 6.5 was maintained by the automatic addition of 10% H_2SO_4 or 10% NaOH. Periodically, 10 mL aseptic samples were taken and extracted with two 10 mL portions of ethyl acetate. The ethyl acetate layer was isolated, dried under a nitrogen stream at 40° C., and the residue was dissolved in 2.0 mL of isopropyl alcohol. The samples were analyzed by reverse phase HPLC (method below) to determine the ratio of compound 472 and the product compound 515. In addition, each sample was analyzed by chiral HPLC (method below) in order to determine the % ee of compound 515. During the bioconversion process, a sterile solution of 30% cerelose and 1.5% yeast extract was fed into the reaction at ~5 mL/hour. After 114 h from the time of substrate addition, reverse phase HPLC analysis indicated the production of a 78% yield of compound 515. Chiral HPLC analysis measured the % e.e. of compound 515 at 94.9%. The above process was repeated in another 3 L bioconversion, and the reaction was conducted at 28° C. at 600 rpm, 1 vvm aeration, with 0.5 g/L of compound 472 input. After 44 h, this reaction gave a 80% yield of compound 515 with 95% e.e. The broth was flittered through a pad of HyFlo™ to provide a clarified fermentation broth. The broth was flittered through a pad of HyFlo™ to provide a clarified fermentation broth. Compound 515 was completely adsorbed onto 55 g of XAD-16 and extracted back into a 1:1 mixture of EtOAc and acetone (3×100 mL) or methyl-tert-butyl ether (3×100 mL). The solvent was removed in vacuo and the resulting residue was purified by silica pad (5 g), eluting with EtOAc. The desired fractions were collected and treated with activated carbon (0.5 g) to declorize the solution and the solvent was removed in vacuo to yield 1.27 g of compound 515. Re-crystallization of this material from EtOAc/heptane (10 mL/20 mL) resulted 950 mg of crystalline compound 515 having 97% purity by reverse phase HPLC and 95% ee by chiral HPLC. Reverse Phase HPLC: YMC Pak ODS-A C18 column, 4.6×50 mm, eluting with a gradient of: 0 min 20% acetonitrile/80% 0.1% TFA in water, 12 min 90% acetonitrile/10% 0.1% TFA in water, 12.01–15 min 20% acetonitrile/80% 0.1% TFA in water, monitoring at 250 nm, 40° C., 5 μL injection volume). Compound 515: RT=8.86 min. Chiral HPLC: CHIRALPAK OD 25×0.46 cm column; isocratic elution with 15% ethanol/85% heptane at 0.5 mL/min, 18° C., monitoring at 220 nm, injection volume: 20 μL. Compound 515: RT=36.5 min.

In an alternate recovery process, the fermentation broth (1L) from the above biohydroxylation reaction was filtered and the cake of cells was washed with 100 mL of water. Clear broth was extracted with ethyl acetate (2×600 mL) and the cake of cells was extracted with 400 mL of ethyl acetate. The combined ethyl acetate layers were concentrated and the resulting residue was dissolved in 5 mL of 1:1 heptane/ethyl acetate and loaded on to silica gel pad pad (70 g in 250 mL fritted glass filter). The silica gel pad was eluted with a gradient of 80 to 90% EtOAc/heptane. Fractions were collected and the fractions containing compound 515 were pooled. The solvent was removed in vacuo and resulting product was crystallized from EtOAc/heptane to give a 90% yield of compound 515 with 98% purity by reverse phase HPLC and 95% ee by chiral HPLC. Reverse Phase HPLC: YMC Pak ODS-A C18 column, 4.6×50 mm, eluting with a gradient of: 0 min 20% acetonitrile/80% 0.1% TFA in water, 12 min 90% acetonitrile/10% 0.1% TFA in water, 12.01–15 min 20% acetonitrile/80% 0.1% TFA in water, monitoring at 250 nm, 40° C., 5 μL injection volume). Compound 472: RT=111.12 min. Compound 515: RT=8.86 min. Chiral HPLC: CHIRALPAK OD 25×0.46 cm column; isocratic elution with 15% ethanol/85% heptane at 0.5 mL/min, 18° C., monitoring at 220 nm, injection volume: 20 μL. Compound 472: RT=27.4 min. Compound 515: RT=36.5 min. Compound (514iii) RT=39.1 min.

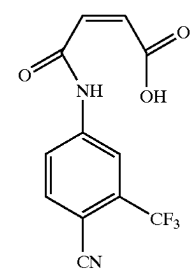
EXAMPLE 516

4-(2,5-Dioxo-2,5-dihydro-pyrrol-1-yl)-2-trifluoromethylbenzonitrile (516B)

The following Example demonstrates preparation of an intermediate useful for preparing compounds of the formula I of the present invention.



A. 3-(4-Cyano-3-trifluoromethylphenylcarbamoyl) acrylic Acid (516A)



5-amino-2-cyanobenzotrifluoride (210.6 mmoles; 40.00 g) and butyl acetate (80 mL) were added to a 250 mL round

bottom flask, followed by the addition of maleic anhydride (231.9 mmoles, 23.20 g). The resulting suspension was heated to 60° C. for 3.5 h. The reaction mixture was cooled to 25° C. and then heptane (160 mL) was added dropwise over a period of 25 minutes. The resulting suspension was filtered and washed with a mixture of 4:1, hepatane:butyl acetate (30 mL) and heptane (45 mL). The cake was dried in vacuo to give 60 g (95% yield) of compound 516A.

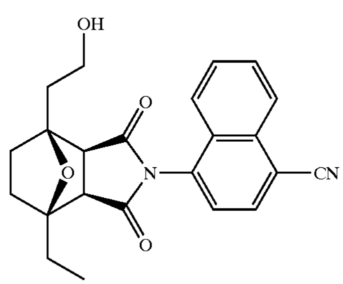
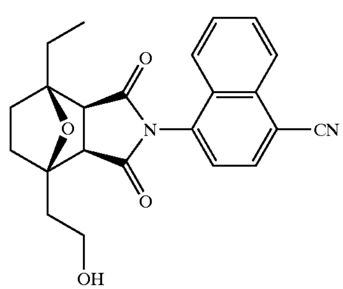
B. 4-(2,5-Dioxo-2,5-dihydro-pyrrol-1-yl)-2-trifluoromethyl-benzonitrile (516B)

Compound 516A (17.42 mmoles, 5.000 g) was added to the reaction flask followed by the addition of zinc bromide (17.58 mmoles, 3.960 g) and then toluene (50.00 mL, 43.25 g) was added to the mixture. The resulting suspension was stirred for 20 min. Hexamethyldisilazane (26.35 mmoles, 5.560 mL, 4.253 g) was added to this suspension which was then heated to 60° C. for 4.5 h. The reaction mixture was diluted with EtOAc (25 mL) and then poured into a 1N HCl solution (30 mL) at 25° C. The organic phase was collected and the aqueous phase was extracted with EtOAc (15 mL). The organic phase was isolated, combined with the earlier organic phase and washed consecutively with saturated $NaHCO_3$ (15 mL), a mixture of 1:1 water:brine solution (15 mL) and brine (15 mL). The resulting solution was dried over $MgSO_4$, filtered and concentrated in vacuo to a 50 mL suspension. Heptane (125 mL) was added dropwise to this suspension with agitation. The resulting thicker suspension was filtered and washed with a mixture of 2:1 heptane:toluene (15 mL) and then heptane (15 mL) to give 4 g (85% yield) of compound 516B. HPLC: 100% at 2.11 min (retention time) (YMC S5 ODS column, 4.6×50 mm, eluting with 10–90% aqueous methanol over 4 min containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

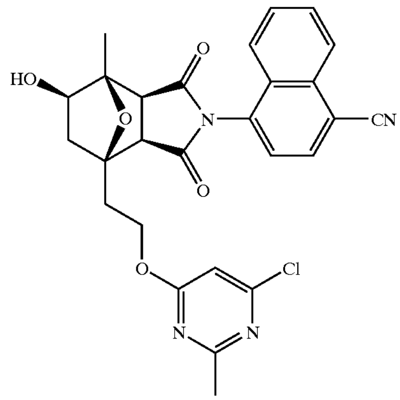
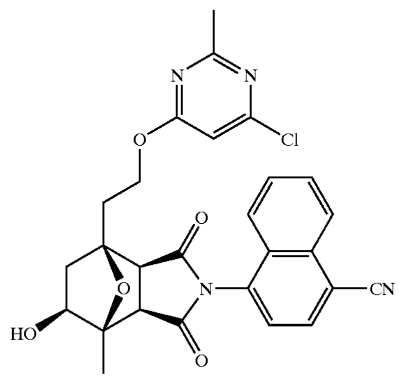
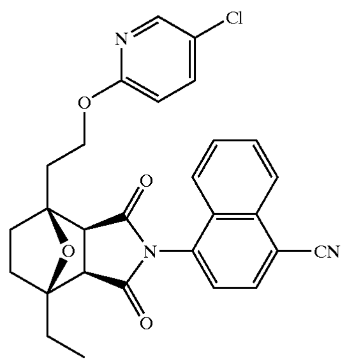
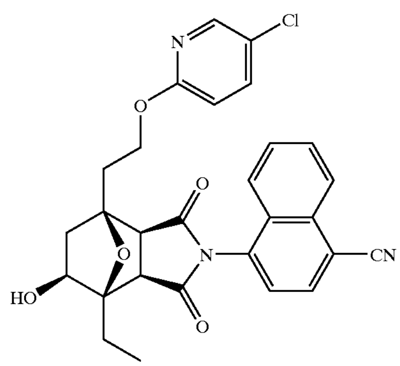
EXAMPLES 517 TO 746 AND 751 TO 753

Additional compounds of the present invention were prepared by procedures analogous to those described above. The compounds of Examples 517 to 746 and 751 to 753 have the structures shown in the following Table 17.

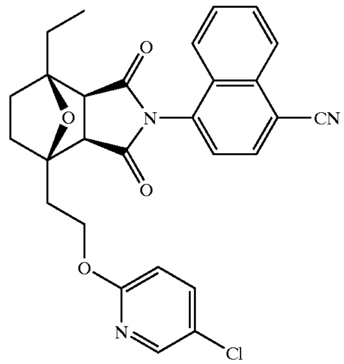
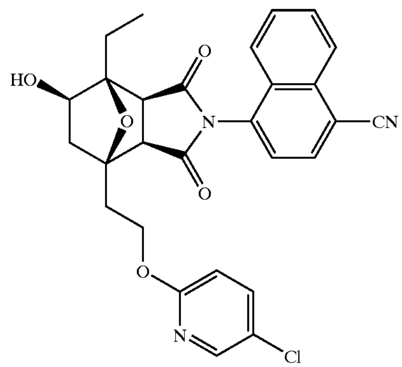
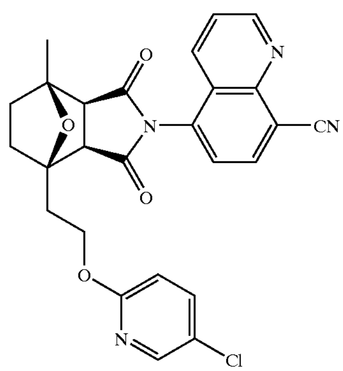
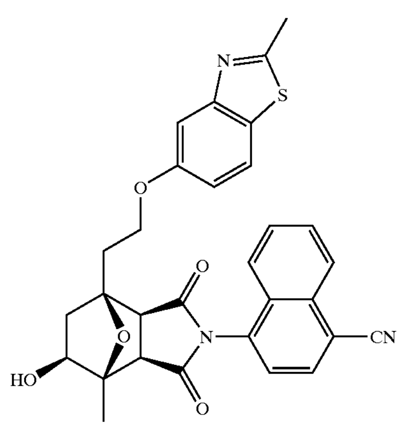
Table 17 also provides the compound name, retention time/molecular mass, and the procedure employed for preparation of these compounds. The chromatography techniques used to determine the compound retention times of Table 17 are as follows: LC and LCMS were described in Examples 439 to 454 (Table 9). The molecular mass of the compounds listed in Table 17, where provided, was determined by MS (ES) by the formula m/z.

Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
517		[3aR-(3aα,4β,7β,7aα)]-4-[4-Ethyloctahydro-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalencarbonitrile	2.84 LC $[M + H]^+$ = 391.16	245 & 461
518		[3aS-(3aα,4β,7β,7aα)]-4-[4-Ethyloctahydro-7-(2-hydroxyethyl)-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.84 LC $[M + H]^+$ = 391.16	245 & 461

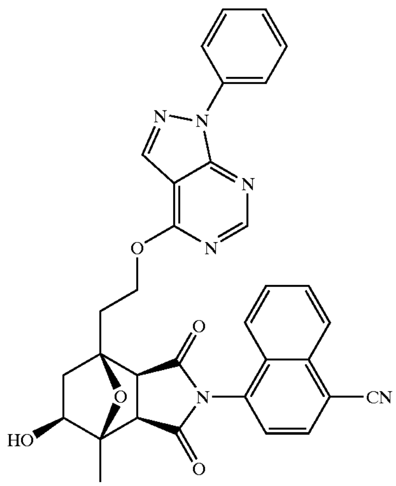
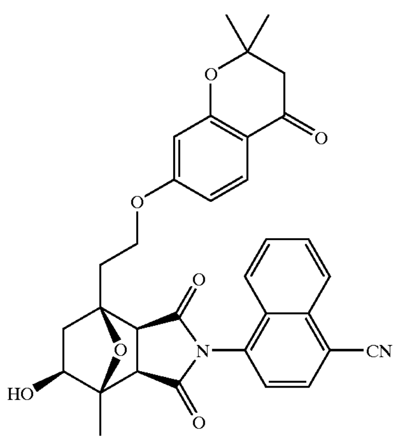
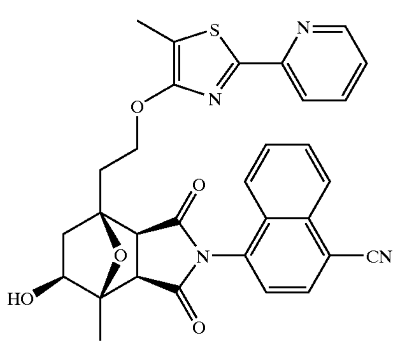
-continued

Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
519		[3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(6-Chloro-2-methyl-4-pyrimidinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.04 LC $[M + H]^+$ = 519.0	243 & 244
520		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(6-Chloro-2-methyl-4-pyrimidinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.04 LC $[M + H]^+$ = 519.0	243 & 244
521		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]-7-ethyloctahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.90 LC $[M + H]^+$ = 502.28	245 & 461
522		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]-4-ethyloctahydro-5-hydroxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.37 LC $[M + H]^+$ = 518.28	435, 499 & 500

-continued

Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
523		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]-7-ethyloctahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.90 LC $[M + H]^+$ = 502.27	245 & 461
524		[3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]-4-ethyloctahydro-5-hydroxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.40 LC $[M + H]^+$ = 518.27	435, 499 & 500
525		(3aα,4β,7β,7aα)-5-[4-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.32 LC $[M + H]^+$ = 489.26	467
526		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-7-[2-[(2-methyl-5-benzothiazolyl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.36 LC $[M + H]^+$ = 540.0	243 & 244

-continued

Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
527		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[(1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.35 LC $[M + OAc]^-$ = 644.8	243 & 244
528		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(3,4-Dihydro-2,2-dimethyl-4-oxo-2H-1-benzopyran-7-yl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.37 LC $[M + OAc]^-$ = 391.16	243 & 244
529		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-7-[2-[[5-methyl-2-(2-pyridinyl)-4-thiazolyl]oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.47 LC $[M + H]^+$ = 566.9	243 & 244

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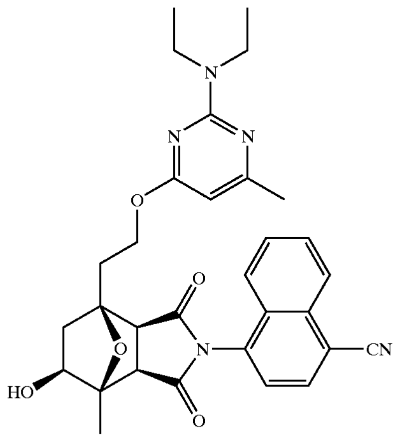
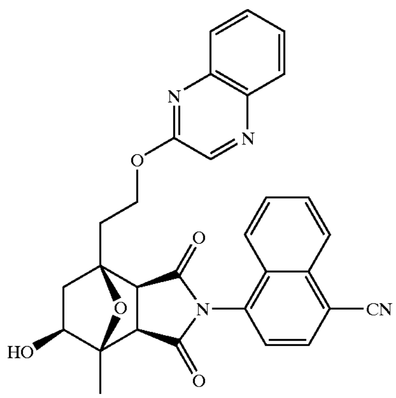
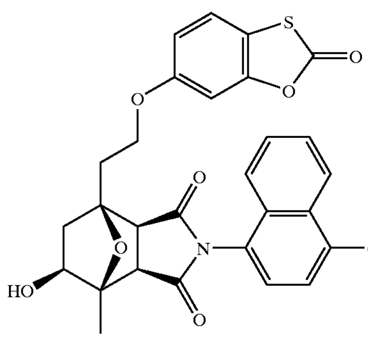
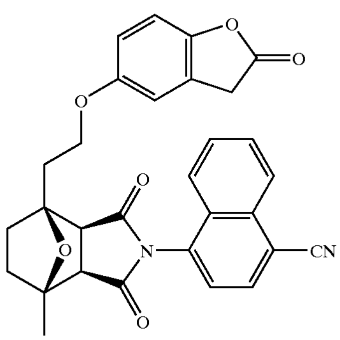
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
530		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[[2-(Diethylamino)-6-methyl-4-pyrimidinyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.47 LC $[M + H]^+$ = 556.1	243 & 244
531		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-(2-quinoxalinyloxy)ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.39 LC $[M + H]^+$ = 520.6	243 & 244
532		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[(2-oxo-1,3-benzoxathiol-5-yl)oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.35 LC $[M + OAc]^-$ = 600.8	243 & 244
533		[3aR-(3aα,4β,7β,7aα)-4-[4-[2-[(2,3-Dihydro-2-oxo-5-benzofuranyl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.28 LC $[M + OAc]^-$ = 566.6	223 & 250

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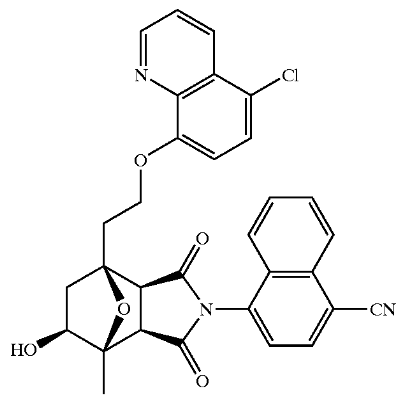
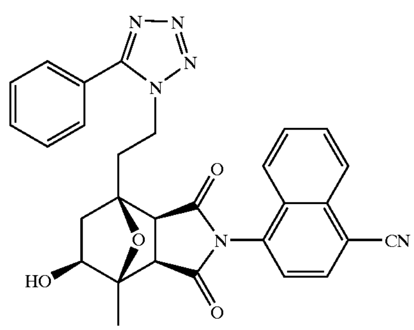
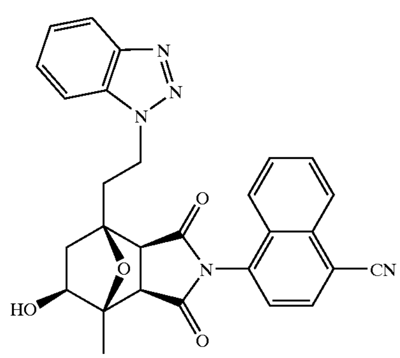
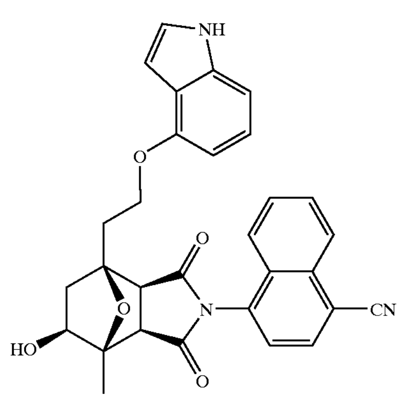
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
534		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-8-quinolinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.18 LC $[M + H]^+$ = 553.6	243 & 244
535		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-(5-phenyl-1H-tetrazol-1-yl)ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.32 LC $[M + H]^+$ = 521.5	243 & 244
536		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(1H-1,2,3-Benzotriazol-1-yl)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.15 LC $[M + H]^+$ = 493.8	243 & 244
537		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-7-[2-(1H-indol-4-yloxy)ethyl]-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.05 LC $[M]^+$ = 507.6	243 & 244

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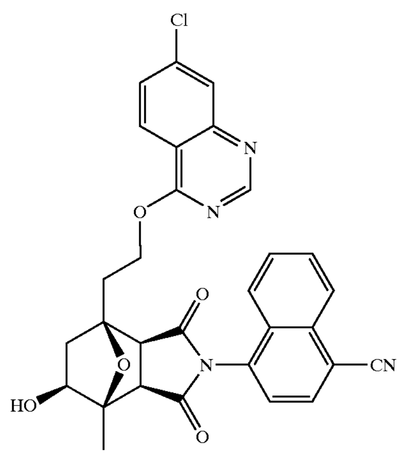
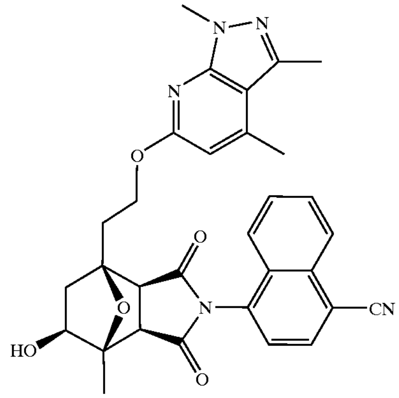
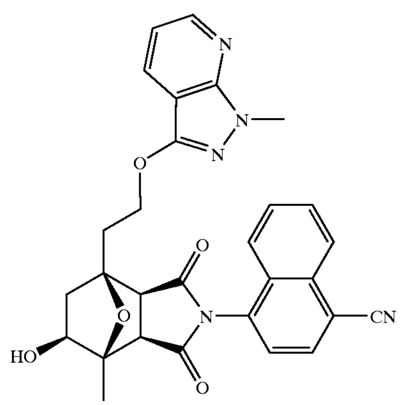
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
538		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(7-Chloro-4-quinazolinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.33 LC $[M + H]^+$ = 555.2	243 & 244
539		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[(1,3,4-trimethyl-1H-pyrazolo[3,4-b]pyridin-6-yl)oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.48 LC $[M + H]^+$ = 552.2	243 & 244
540		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-7-[2-[(1-methyl-1H-pyrazolo[3,4-b]pyridin-3-yl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.08 LC $[M + H]^+$ = 524.2	243 & 244

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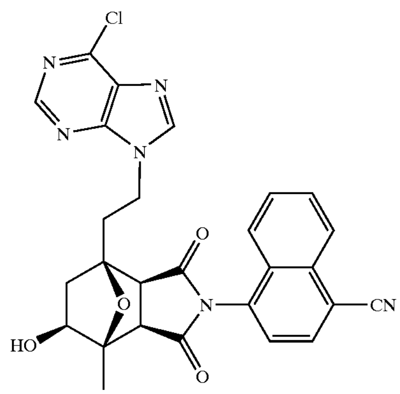
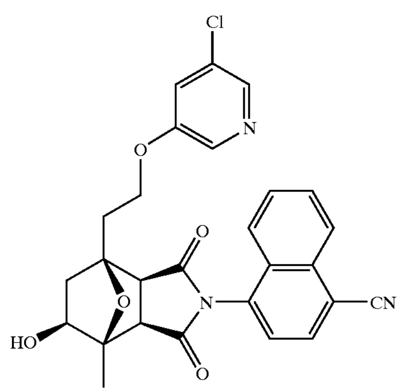
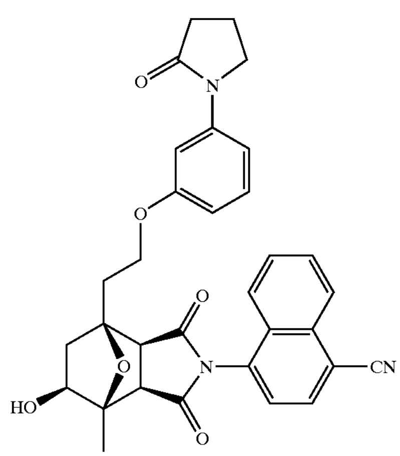
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
541		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(6-Chloro-9H-purin-9-yl)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.70 LC $[M + H]^+$ = 529.0	243 & 244
542		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-3-pyridinyl)oxy]ethyl] octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.17 LC $[M + H]^+$ = 504.1	243 & 244
543		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[3-(2-oxo-1-pyrrolidinyl)phenoxy] ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.10 LC $[M + H]^+$ = 552.3	243 & 244

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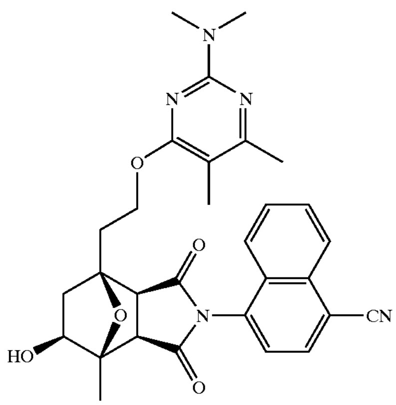
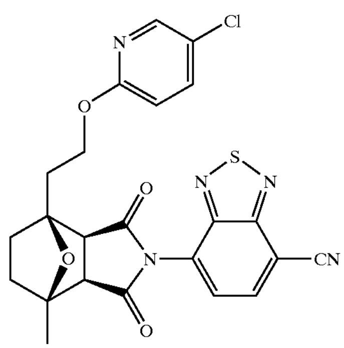
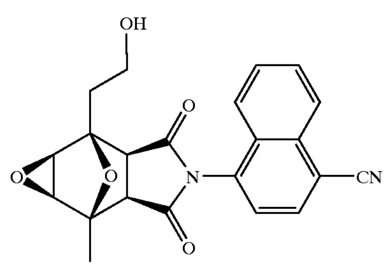
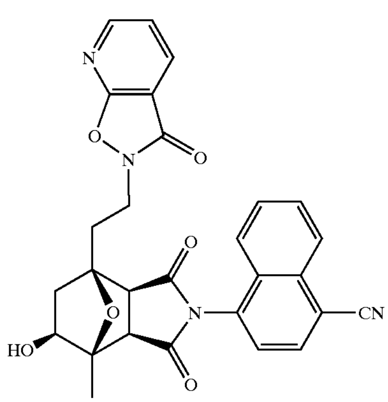
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
544		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[[2-(Dimethylamino)-5,6-dimethyl-4-pyrimidinyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.40 LC $[M + H]^+$ = 542.3	243 & 244
545		(3aα,4β,7β,7aα)-7-[4-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile	3.52 LC $[M + H]^+$ = 495.6	424A, 204, 482F & 482G
546		(1aα,2β,2aα,5aα,6β,6aα)-4-[Octahydro-2-(2-hydroxyethyl)-6-methyl-3,5-dioxo-2,6-epoxy-4H-oxireno[f]isoindol-4-yl]-1-naphthalenecarbonitrile	2.36 LC $[M + H]^+$ = 391.31	460 & 228
547		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-(3-oxoisoxazolo[5,4-b]pyridin-2(3H)-yl)ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.92 LC $[M + OAc]^-$ = 568.6	243 & 244

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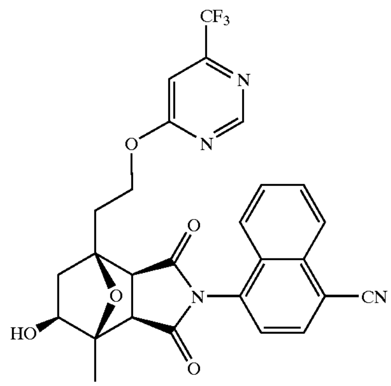
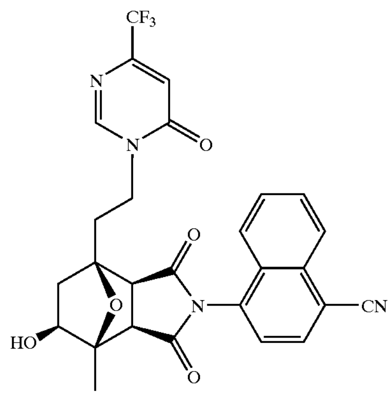
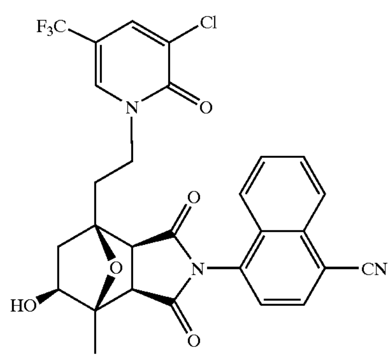
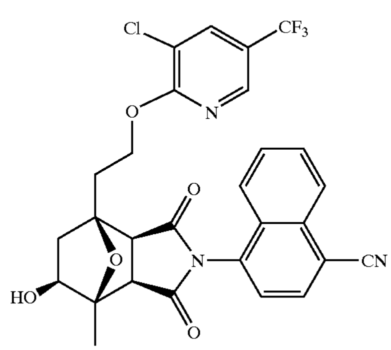
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
548		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[[6-(trifluoromethyl)-4-pyrimidinyl]oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.18 LC $[M + OAc]^-$ = 596.7	243 & 244
549		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[6-oxo-4-(trifluoromethyl)-1(6H)-pyrimidinyl]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.94 LC $[M + OAc]^-$ = 596.5	243 & 244
550		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[3-Chloro-2-oxo-5-(trifluoromethyl)-1(2H)-pyridinyl]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.39 LC $[M + OAc]^-$ = 629.3	243 & 244
551		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[[3-Chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.76 LC $[M + OAc]^-$ = 629.6	243 & 244

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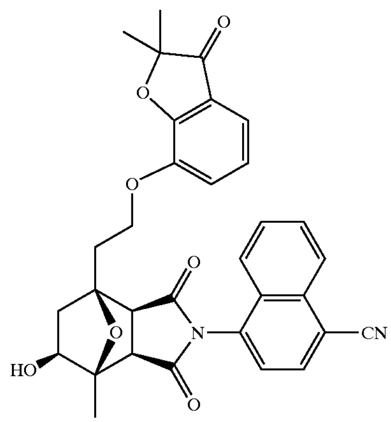
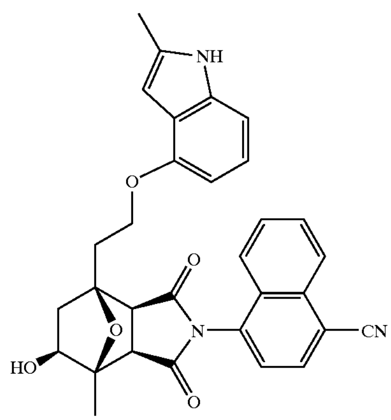
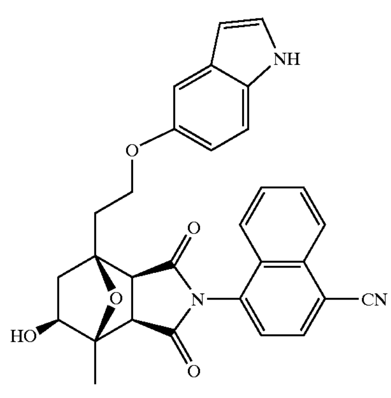
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
552		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(2,3-Dihydro-2,2-dimethyl-3-oxo-7-benzofuranyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.26 LC $[M + OAc]^-$ = 611.5	243 & 244
553		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-7-[2-[(2-methyl-1H-indol-4-yl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.16 LC $[M + H]^+$ = 522.5	243 & 244
554		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-7-[2-(1H-indol-5-yloxy)ethyl]-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.03 LC $[M + H]^+$ = 506.3	243 & 244

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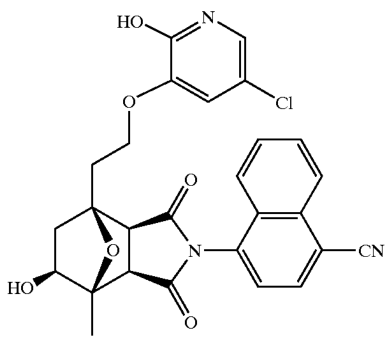
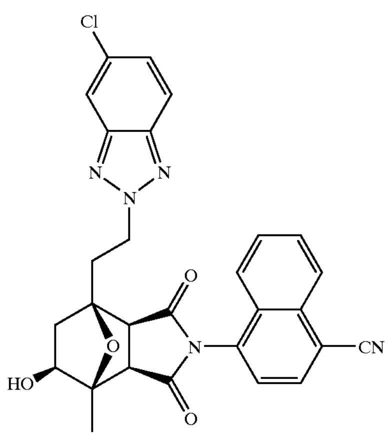
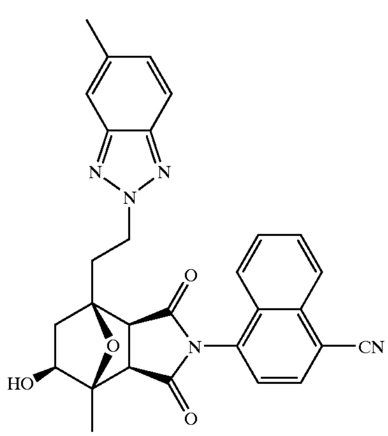
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
555		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-1,2-dihydro-2-oxo-3-pyridinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.85 LC $[M + H]^+$ = 520.5	243 & 244
556		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(5-Chloro-2H-1,2,3-benzotriazol-2-yl)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.53 LC $[M + OAc]^-$ = 586.3	243 & 244
557		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-7-[2-(5-methyl-2H-1,2,3-benzotriazol-2-yl)ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.35 LC $[M + H]^+$ = 508.5	243 & 244

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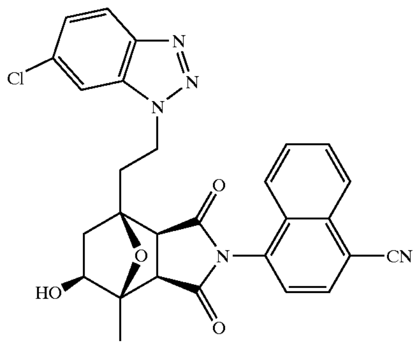
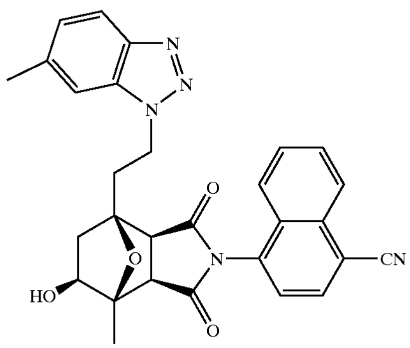
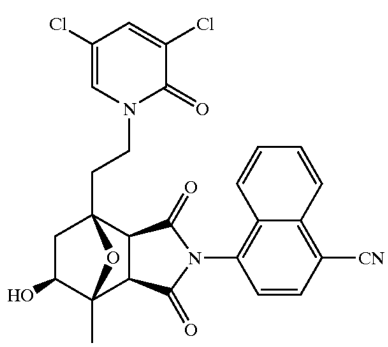
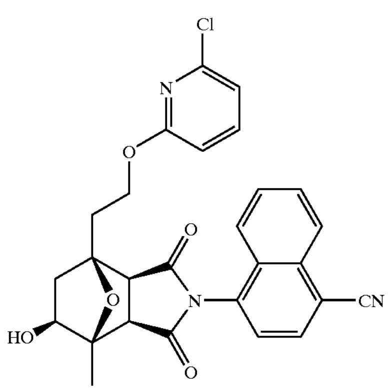
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
558		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(6-Chloro-1H-1,2,3-benzotriazol-1-yl)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.32 LC $[M + H]^+$ = 528.3	243 & 244
559		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-4-methyl-7-[2-(6-methyl-1H-1,2,3-benzotriazol-1-yl)ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.97 LC $[M + H]^+$ = 508.4	243 & 244
560		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(3,5-Dichloro-2-oxo-1(2H)-pyridinyl)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.22 LC $[M + H]^+$ = 538.3	243 & 244
561		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(6-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.30 LC $[M + H]^+$ = 504.3	243 & 244

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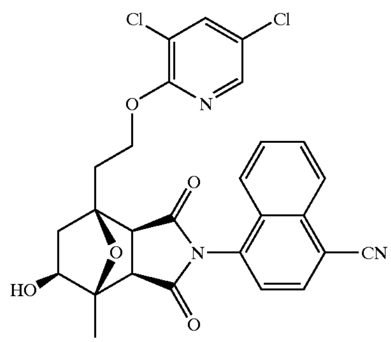
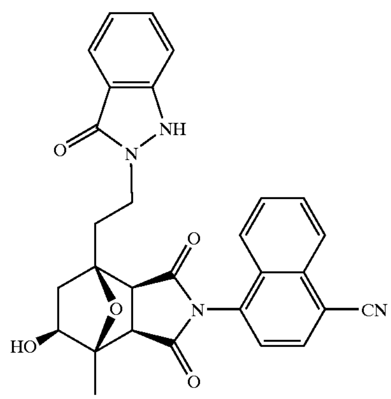
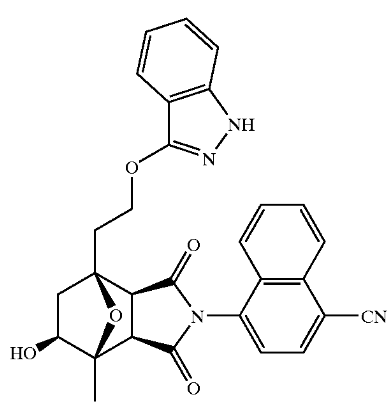
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
562		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(3,5-Dichloro-2-pyridinyl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.67 LC $[M + H]^+$ = 596.3	243 & 244
563		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(1,3-Dihydro-3-oxo-2H-indazol-2-yl)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.82 LC $[M + H]^+$ = 509.1	243 & 244
564		[3aR-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-hydroxy-7-[2-(1H-indazol-3-yloxy)ethyl]-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.19 LC $[M + H]^+$ = 509.2	243 & 244

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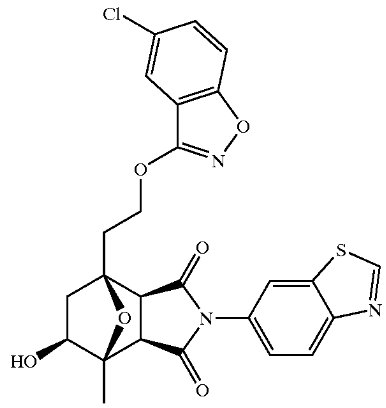
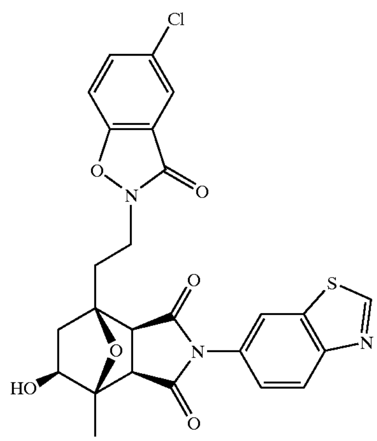
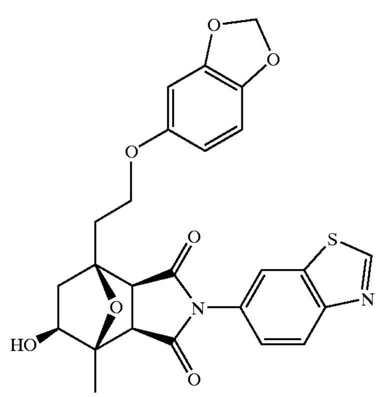
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
565		[3aR-(3aα,4β,5β,7β,7aα)]-2-(6-Benzothiazolyl)-7-[2-[(5-chloro-1,2-benzisoxazol-3-yl)oxy]ethyl]hexahydro-5-hydroxy-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.21 LC $[M + H]^+$ = 526.2	481
566		[3aR-(3aα,4β,5β,7β,7aα)]-2-(6-Benzothiazolyl)-7-[2-(5-chloro-3-oxo-1,2-benzisoxazol-2(3H)-yl)ethyl]hexahydro-5-hydroxy-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.81 LC $[M + H]^+$ = 526.2	481
567		[3aR-(3aα,4β,5β,7β,7aα)]-7-[2-(1,3-Benzodioxol-5-yloxy)ethyl]-2-(6-benzothiazolyl)hexahydro-5-hydroxy-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.80 LC $[M + H]^+$ = 495.2	481

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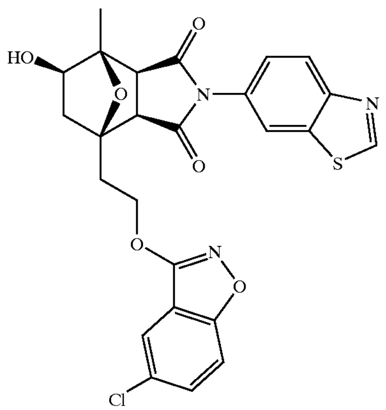
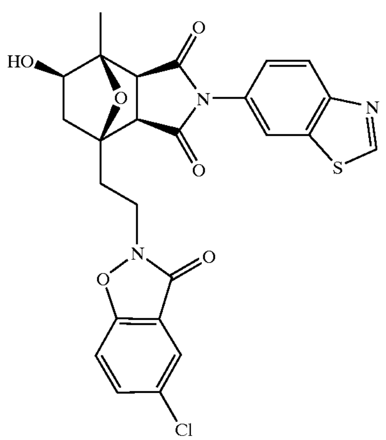
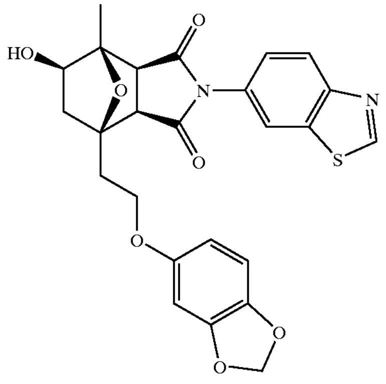
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
568		[3aS-(3aα,4β,5β,7β,7aα)]-2-(6-Benzothiazolyl)-7-[2-[(5-chloro-1,2-benzisoxazol-3-yl)oxy]ethyl]hexahydro-5-hydroxy-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	3.21 LC $[M + H]^+$ = 526.1	481
569		[3aS-(3aα,4β,5β,7β,7aα)]-2-(6-Benzothiazolyl)-7-[2-(5-chloro-3-oxo-1,2-benzisoxazol-2(3H)-yl)ethyl]hexahydro-5-hydroxy-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.84 LC $[M + H]^+$ = 391.16	481
570		[3aS-(3aα,4β,5β,7β,7aα)]-7-[2-(1,3-Benzodioxol-5-yloxy)ethyl]-2-(6-benzothiazolyl)hexahydro-5-hydroxy-4-methyl-4,7-epoxy-1H-isoindole-1,3(2H)-dione	2.82 LC $[M + H]^+$ = 495.2	481

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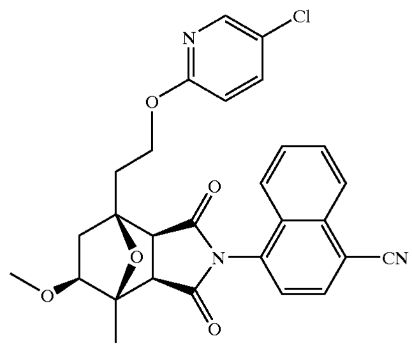
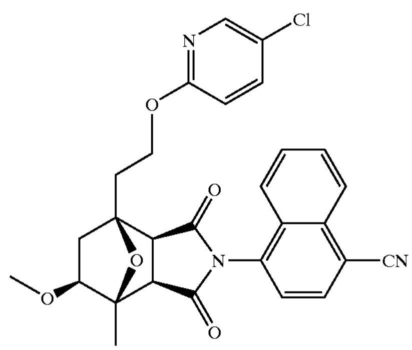
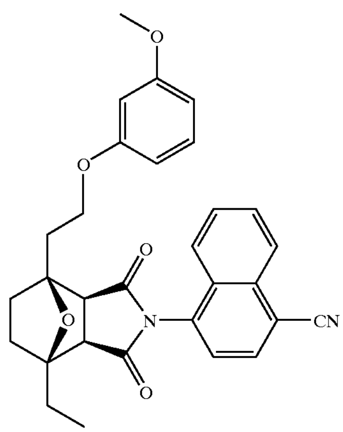
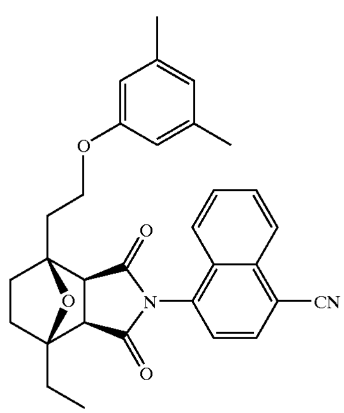
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
571		(3aα,4β,5β,7β,7aα)-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-methoxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.64 & 3.76 atropisomers LCMS $[M + H]^+$ = 518.19	491
572		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-5-methoxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.50 LC $[M + H]^+$ = 518.28	491
573		[3aR-(3aα,4β,7β,7aα)]-4-[4-Ethyloctahydro-7-[2-(3-methoxyphenoxy)ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	7.49 & 7.75 atropisomers LC HRMS $[M + CH_3CO_2]^-$ = 555.2144	245C, 461 & 462
574		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(3,5-Dimethylphenoxy)ethyl]-7-ethyloctahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	8.10 & 8.31 atropisomers HRMS $[M + CH_3CO_2]^-$ = 553.2363	245C, 461 & 462

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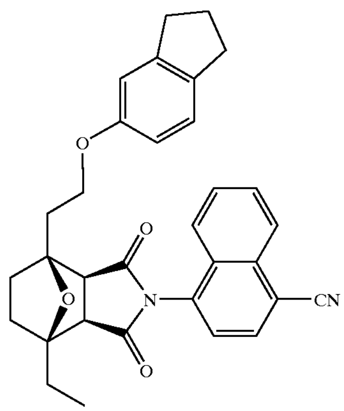
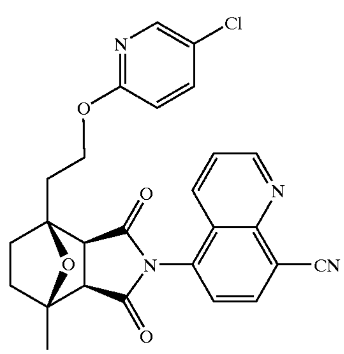
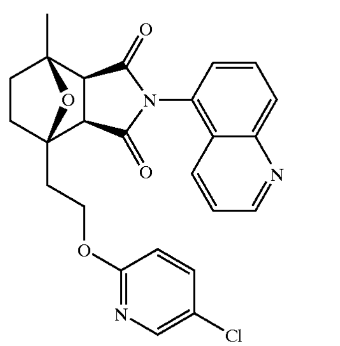
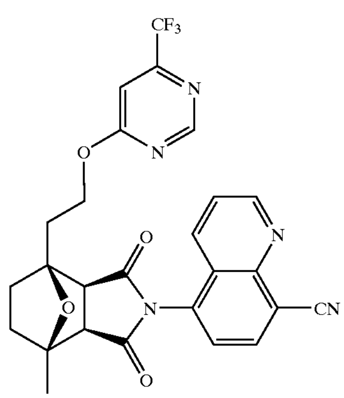
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
575		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-[(2,3-Dihydro-1H-inden-5-yl)oxy]ethyl]-7-ethyloctahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	8.17 & 8.37 atropisomers HRMS $[M + CH_3CO_2]^-$ = 565.2326	245C, 461 & 462
576		[3aR-(3aα,4β,7β,7aα)]-5-[4-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.45 LC $[M + H]^+$ = 489.0	467 & 487
577		[3aS-(3aα,4β,7β,7aα)]-5-[4-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.45 LC $[M + H]^+$ = 488.99	467 & 487
578		[3aR-(3aα,4β,7β,7aα)]-5-[Octahydro-4-methyl-1,3-dioxo-7-[2-[[6-(trifluoromethyl)-4-pyrimidinyl]oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.25 LC $[M + H]^+$ = 524.0	467 & 487

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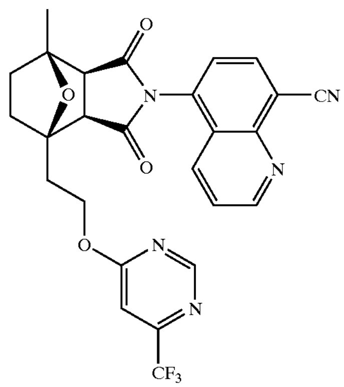
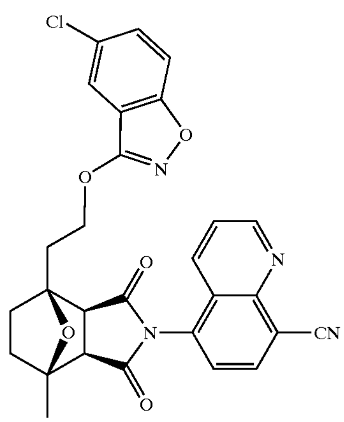
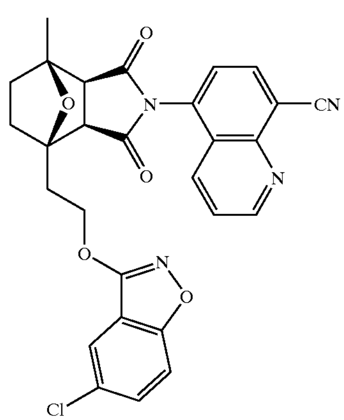
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
579		[3aS-(3aα,4β,7β,7aα)]-5-[Octahydro-4-methyl-1,3-dioxo-7-[2-[[6-(trifluoromethyl)-4-pyrimidinyl]oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.45 LC $[M + H]^+$ = 523.98	467 & 487
580		[3aR-(3aα,4β,7β,7aα)]-5-[4-[2-[(5-Chloro-1,2-benzisoxazol-3-yl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.66 LC $[M + H]^+$ = 529.16	467 & 487
581		[3aS-(3aα,4β,7β,7aα)]-5-[4-[2-[(5-Chloro-1,2-benzisoxazol-3-yl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.66 LC $[M + H]^+$ = 529.16	467 & 487

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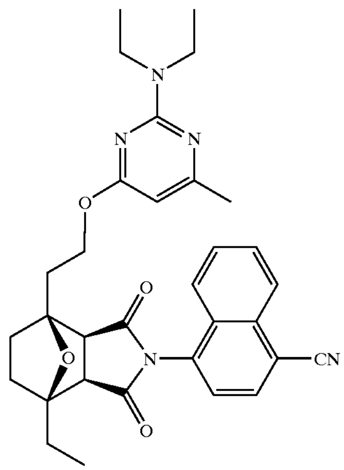
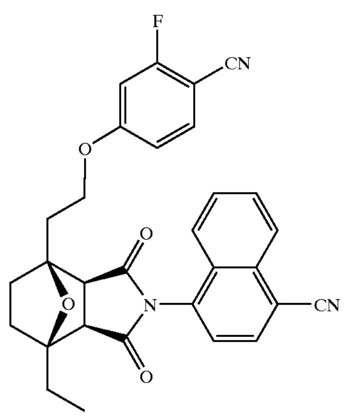
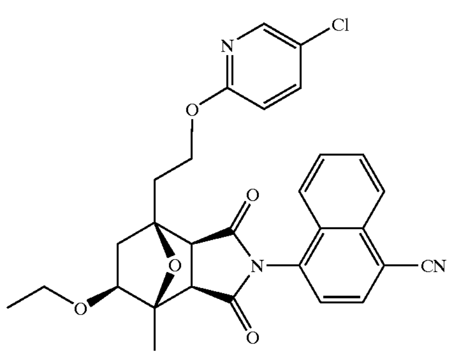
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
582		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-[[2-(Diethylamino)-6-methyl-4-pyrimidinyl]oxy]ethyl]-7-ethyloctahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	5.85 & 6.06 atropisomers LC $[M + H]^+$ = 554.26	245C, 461 & 462
583		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(4-Cyano-3-fluorophenoxy)ethyl]-7-ethyloctahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	7.23 & 7.50 atropisomers HRMS $[M - H]^-$ = 508.1682	245C, 461 & 462
584		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]-5-ethoxyoctahydro-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.60 LC $[M + H]^+$ = 532.23	223, 495 & 496

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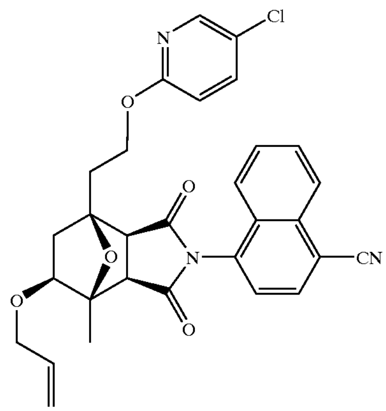
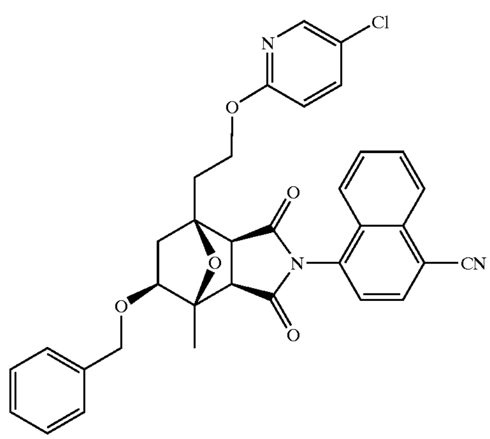
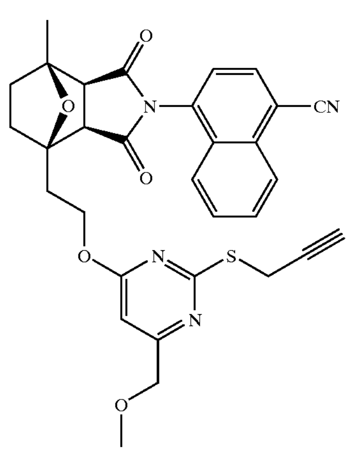
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
585		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-4-methyl-1,3-dioxo-5-(2-propenyloxy)-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.68 LC $[M + H]^+$ = 544.23	491
586		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-4-methyl-1,3-dioxo-5-(phenylmethoxy)-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.63 LC $[M + H]^+$ = 594.26	491
587		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-[[6-(methoxymethyl)-2-(2-propynylthio)-4-pyrimidinyl]oxy]ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.02 LC $[M + H]^+$ = 567.31	491

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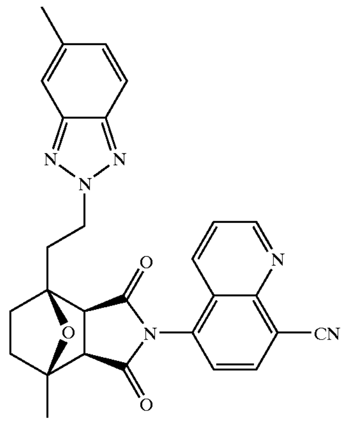
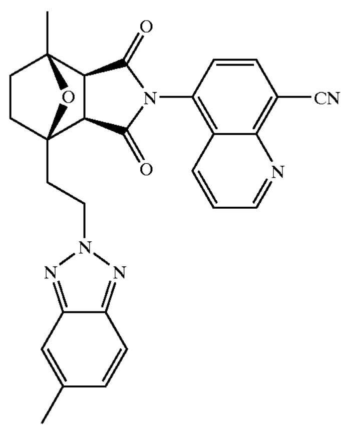
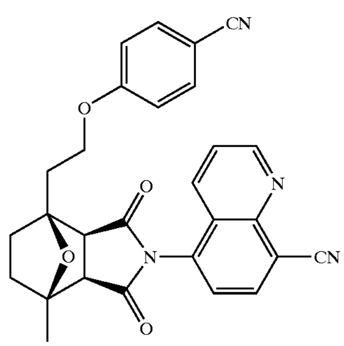
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
588		[3aR-(3aα,4β,7β,7aα)]-5-[Octahydro-4-methyl-7-[2-(5-methyl-2H-1,2,3-benzotriazol-2-yl)ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.37 LC $[M + H]^+$ = 493.24	467 & 487
589		[3aS-(3aα,4β,7β,7aα)]-5-[Octahydro-4-methyl-7-[2-(5-methyl-2H-1,2,3-benzotriazol-2-yl)ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.37 LC $[M + H]^+$ = 493.24	467 & 487
590		[3aR-(3aα,4β,7β,7aα)]-5-[4-[2-(4-Cyanophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.14 LC $[M + H]^+$ = 479.22	467 & 487

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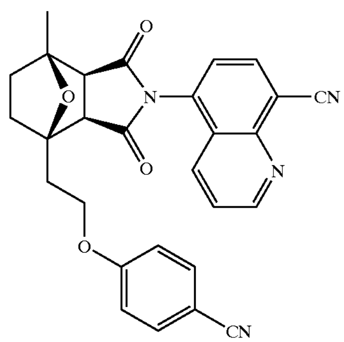
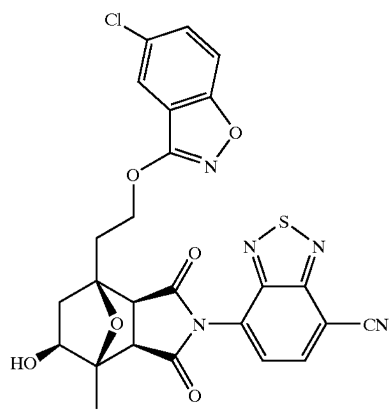
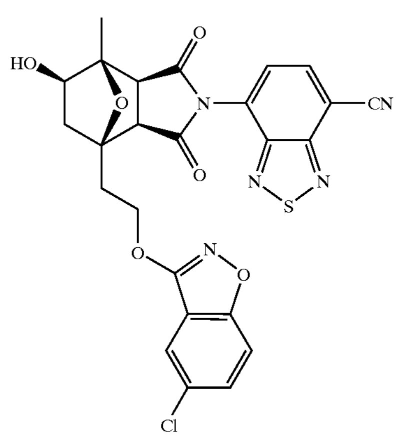
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
591		[3aS-(3aα,4β,7β,7aα)]-5-[4-[2-(4-Cyanophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.14 LC $[M + H]^+$ = 479.22	467 & 487
592		[3aR-(3aα,4β,5β,7β,7aα)]-7-[7-[2-[(5-Chloro-1,2-benzisoxazol-3-yl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile	3.38 LC $[M + H]^+$ = 552.12	482
593		[3aS-(3aα,4β,5β,7β,7aα)]-7-[7-[2-[(5-Chloro-1,2-benzisoxazol-3-yl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile	3.39 LC $[M + H]^+$ = 552.10	482

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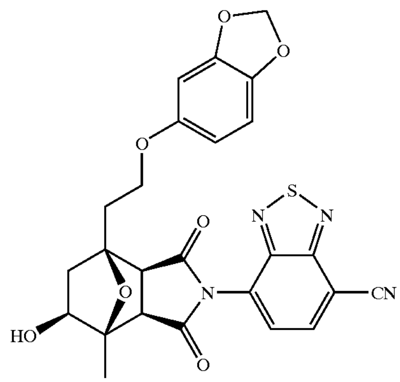
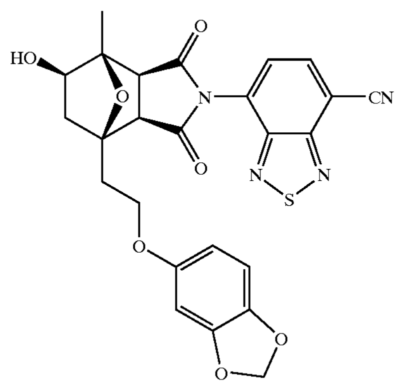
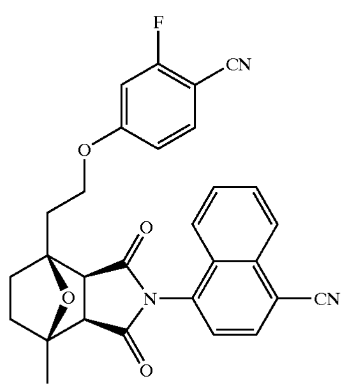
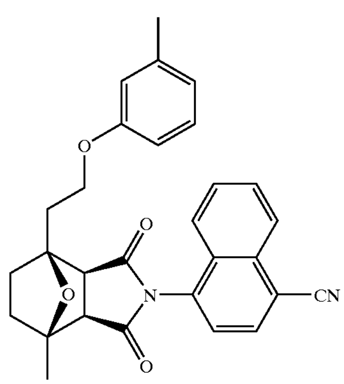
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
594		[3aR-(3aα,4β,5β,7β,7aα)]-7-[7-[2-(1,3-Benzodioxol-5-yloxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile	3.00 LC $[M + H]^+$ = 521.15	482
595		[3aS-(3aα,4β,5β,7β,7aα)]-7-[7-[2-(1,3-Benzodioxol-5-yloxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2,1,3-benzothiadiazole-4-carbonitrile	2.99 LC $[M + H]^+$ = 521.14	482
596		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(4-Cyano-3-fluorophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.76 LCMS $[M + H]^+$ = 496.2	496
597		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-(3-methylphenoxy)ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.79 LCMS $[M + H]^+$ = 467.2	496

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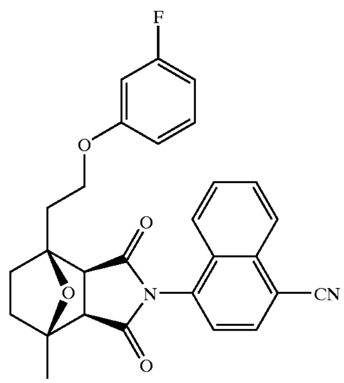
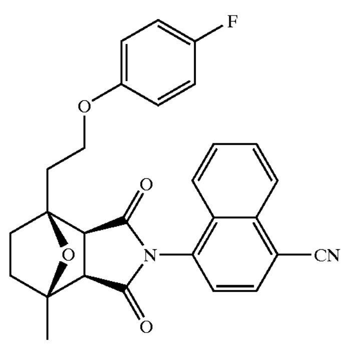
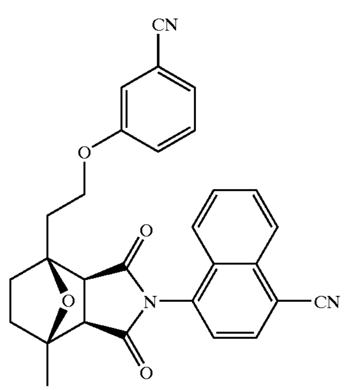
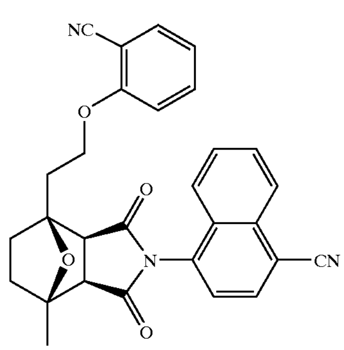
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
598		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(3-Fluorophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.01 LCMS $[M + H]^+$ = 471.2	496
599		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(4-Fluorophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.98 LCMS $[M + H]^+$ = 471.2	496
600		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(3-Cyanophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.88 LCMS $[M + H]^+$ = 478.2	496
601		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(2-Cyanophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.42 LCMS $[M + H]^+$ = 478.2	496

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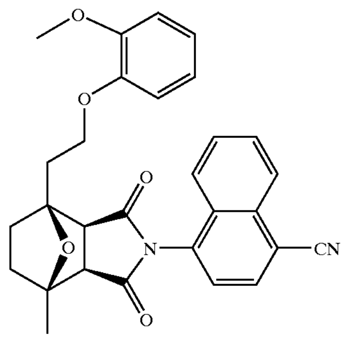
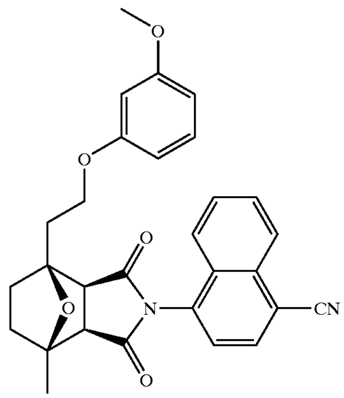
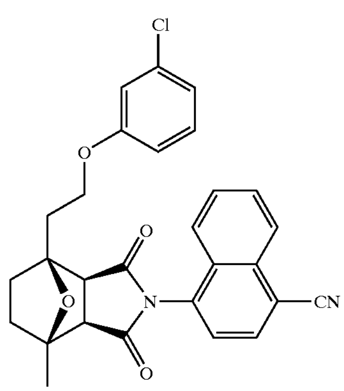
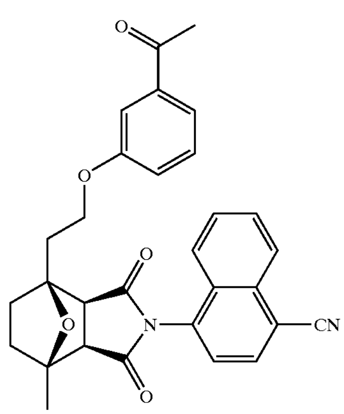
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
602		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-(2-methoxyphenoxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.83 LCMS $[M + H]^+$ = 483.2	496
603		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-(3-methoxyphenoxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.14 LCMS $[M + H]^+$ = 483.2	496
604		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(3-Chlorophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.09 LCMS $[M + H]^+$ = 487.2	496
605		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(3-Acetylphenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.20 LCMS $[M + H]^+$ = 495.2	496

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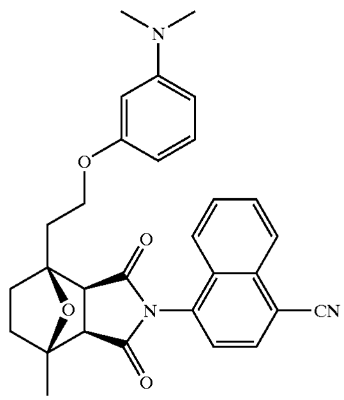
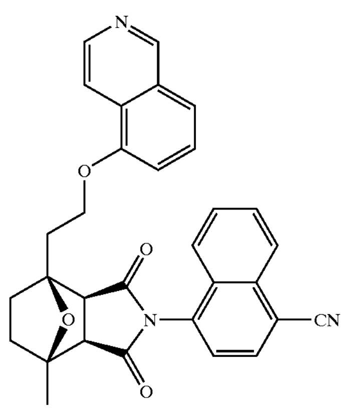
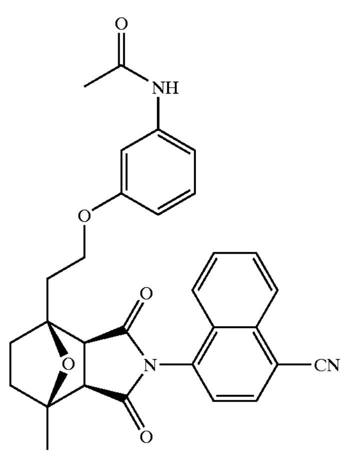
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
606		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-[3-(Dimethylamino)phenoxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.94 LCMS $[M + H]^+$ = 496.2	496
607		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-(5-isoquinolinyloxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.89 LCMS $[M + H]^+$ = 504.2	496
608		N-[3-[2-[3aR-(3aα,4β,7β,7aα)]-2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy]phenylacetamide	1.97 LCMS $[M + H]^+$ = 510.6	496

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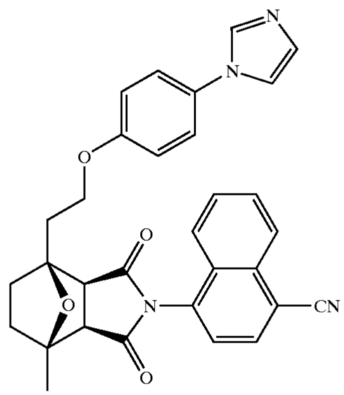
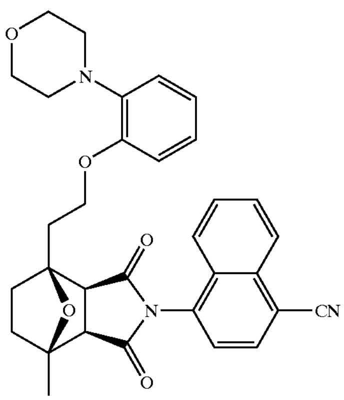
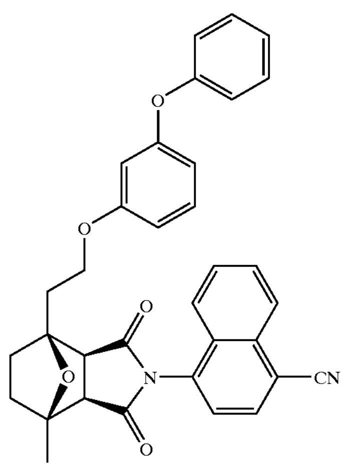
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
609		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-[4-(1H-imidazol-1-yl)phenoxy]ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.81 LCMS $[M + H]^+$ = 519.6	496
610		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-[2-(4-morpholinyl)phenoxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.16 LCMS $[M + H]^+$ = 538.2	496
611		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-(3-phenoxyphenoxy)ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.89 LCMS $[M + H]^+$ = 545.2	496

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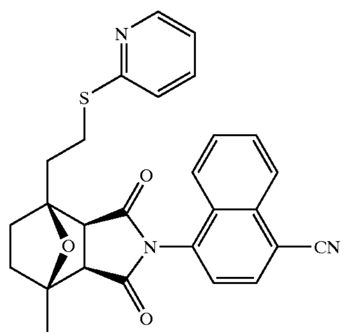
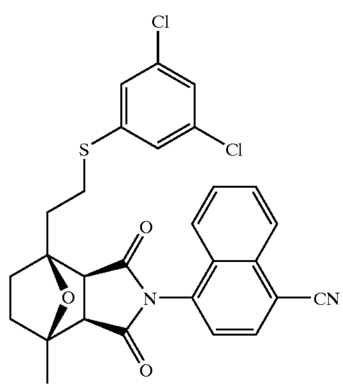
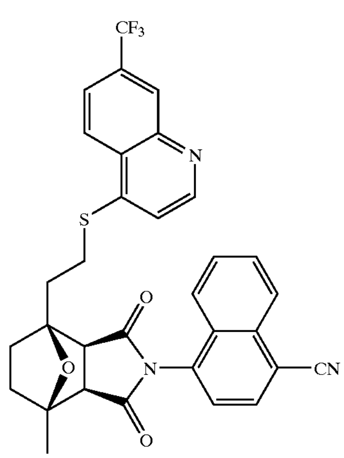
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
612		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-(2-pyridinylthio)ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.91 LCMS $[M + H]^+$ = 469.6	496
613		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-[(3,5-Dichlorophenyl)thio]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.53 LCMS $[M + H]^+$ = 537.1	496
614		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[[7-(trifluoromethyl)-4-quinolinyl]thio]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.49 LCMS $[M + H]^+$ = 588.2	496

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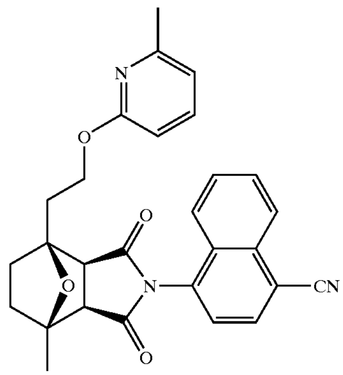
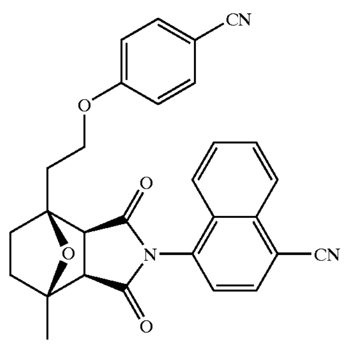
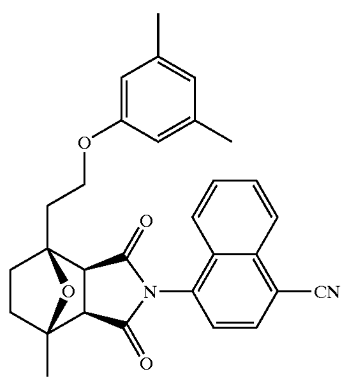
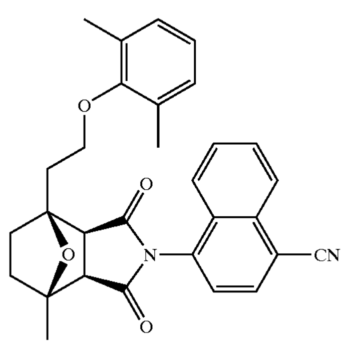
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
615		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-[(6-methyl-2-pyridinyl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.79 LCMS $[M + H]^+$ = 467.5	496
616		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(4-Cyanophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.98 LCMS $[M + H]^+$ = 478.2	496
617		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(3,5-Dimethylphenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.80 LCMS $[M + H]^+$ = 481.2	496
618		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(2,6-Dimethylphenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.88 LCMS $[M + H]^+$ = 481.2	496

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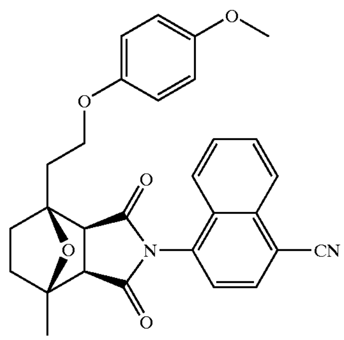
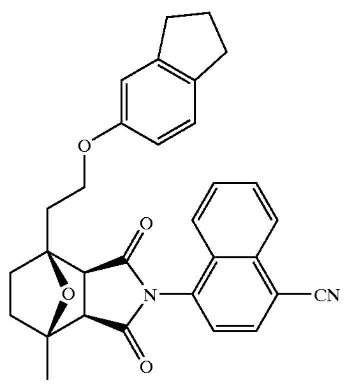
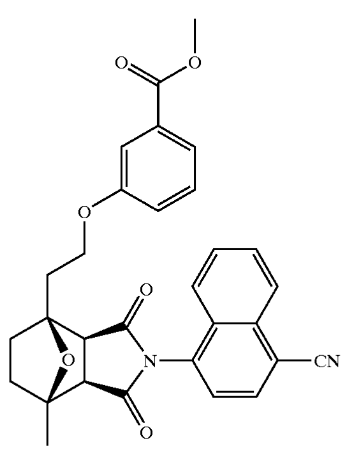
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
619		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-(4-methoxyphenoxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.50 LCMS $[M + H]^+$ = 483.2	496
620		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-[(2,3-Dihydro-1H-inden-5-yl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.14 LCMS $[M + H]^+$ = 493.3	496
621		[3-[2-[[3aR-(3aα,4β,7β,7aα)]-2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy]benzoic acid, methyl ester	3.99 LCMS $[M + H]^+$ = 511.2	496

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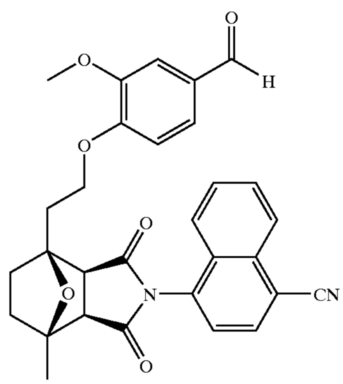
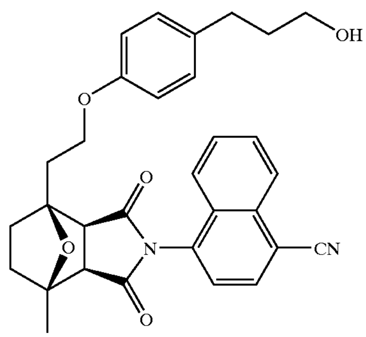
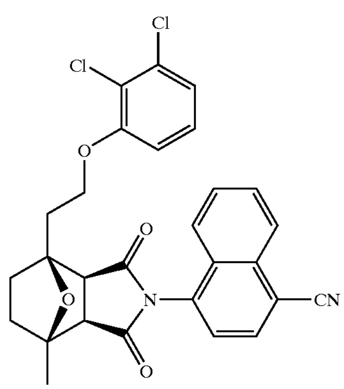
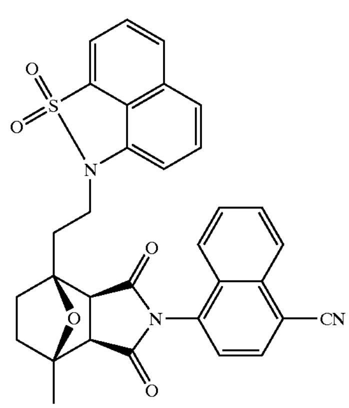
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
622		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(4-Formyl-2-methoxyphenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.01 LCMS $[M + H]^+$ = 511.2	496
623		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-[4-(3-hydroxypropyl)phenoxy]ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.93 LCMS $[M + H]^+$ = 511.3	496
624		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(2,3-Dichlorophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.79 LCMS $[M + H]^+$ = 521.1	496
625		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(1,1-Dioxido-2H-naphtho[1,8-cd]isothiazol-2-yl)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.69 LCMS $[M + H]^+$ = 564.2	496

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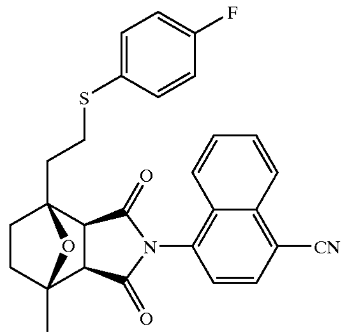
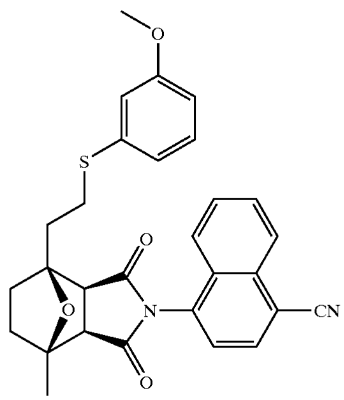
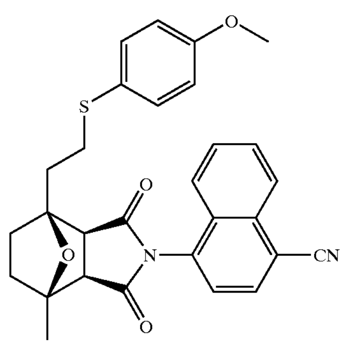
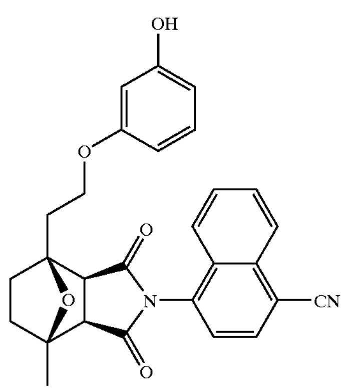
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
626		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-[(4-Fluorophenyl)thio]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.03 LCMS $[M + H]^+$ = 487.2	496
627		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-[(3-methoxyphenyl)thio]ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.02 LCMS $[M + H]^+$ = 499.2	496
628		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-[(4-methoxyphenyl)thio]ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	1.99 LCMS $[M + H]^+$ = 499.2	496
629		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-(3-hydroxyphenoxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.27 LCMS $[M + H]^+$ = 469.2	496

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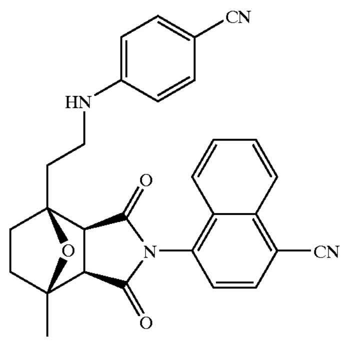
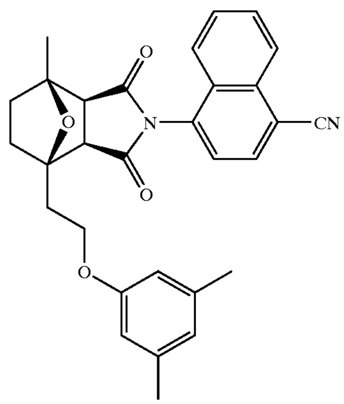
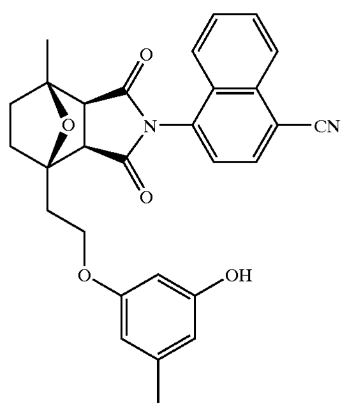
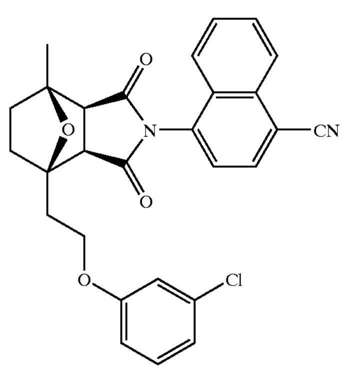
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
630		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-[(4-Cyanophenyl)amino]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.56 LCMS $[M + H]^+$ = 477.2	496
631		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(3,5-Dimethylphenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.10 LCMS $[M + H]^+$ = 481.2	496
632		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-(3-hydroxy-5-methylphenoxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.56 LCMS $[M + H]^+$ = 483.2	496
633		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(3-Chlorophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.03 LCMS $[M + H]^+$ = 487.1	496

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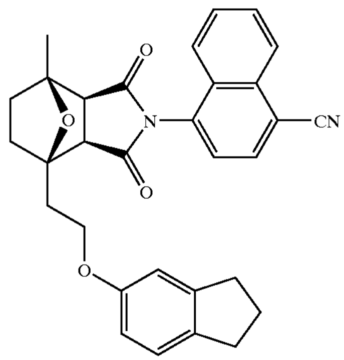
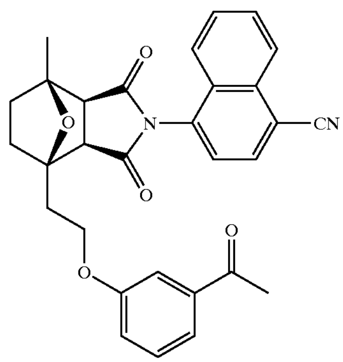
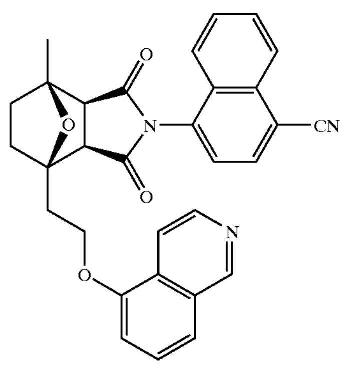
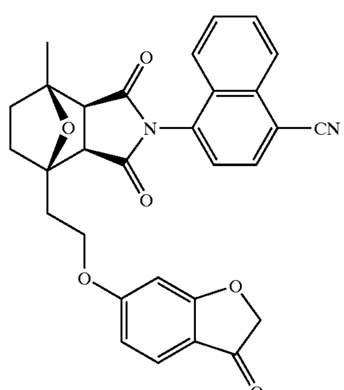
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
634		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[(2,3-Dihydro-1H-inden-5-yl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.15 LCMS $[M + H]^+$ = 493.2	496
635		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(3-Acetylphenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.65 LCMS $[M + H]^+$ = 495.2	496
636		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-(5-isoquinolinyloxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	3.67 LCMS $[M + H]^+$ = 504.2	496
637		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[(2,3-Dihydro-3-oxo-6-benzofuranyl)oxy]ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.90 LCMS $[M + H]^+$ = 509.2	496

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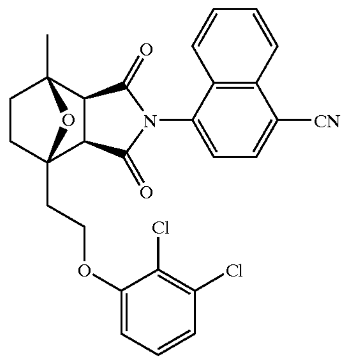
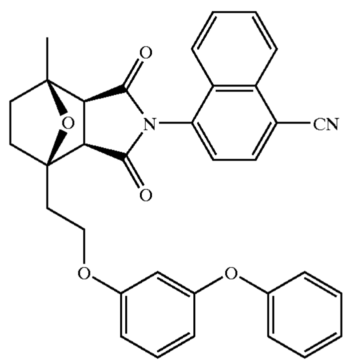
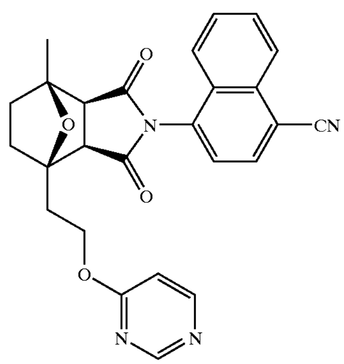
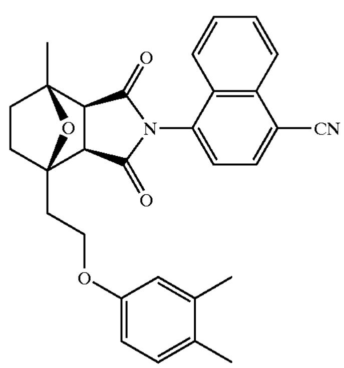
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
638		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(2,3-Dichlorophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.80 LCMS $[M + H]^+$ = 521.1	496
639		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-(3-phenoxyphenoxy)ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.54 LCMS $[M + H]^+$ = 545.2	496
640		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-(4-pyrimidinyloxy)ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.13 LCMS $[M + H]^+$ = 455.2	496
641		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(3,4-Dimethylphenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.19 LCMS $[M + H]^+$ = 481.2	496

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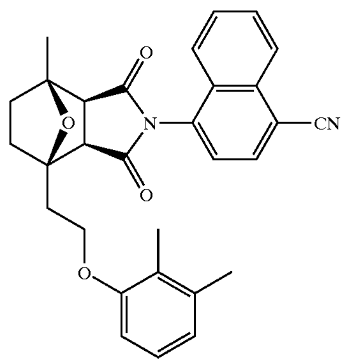
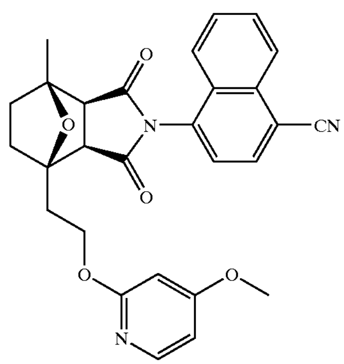
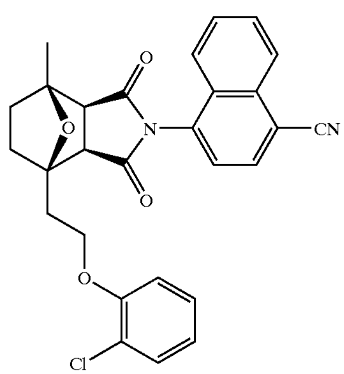
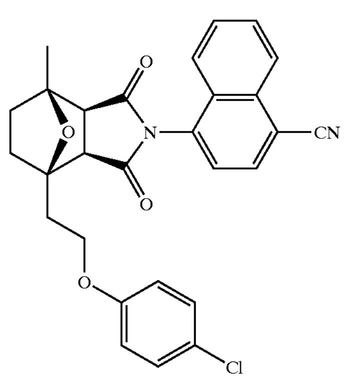
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
642		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(2,3-Dimethylphenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.80 LCMS $[M + H]^+$ = 481.2	496
643		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-[(4-methoxy-2-pyridinyl)oxy]ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.72 LCMS $[M + H]^+$ = 484.2	496
644		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(2-Chlorophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.63 LCMS $[M + H]^+$ = 487.1	496
645		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(4-Chlorophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.07 LCMS $[M + H]^+$ = 487.1	496

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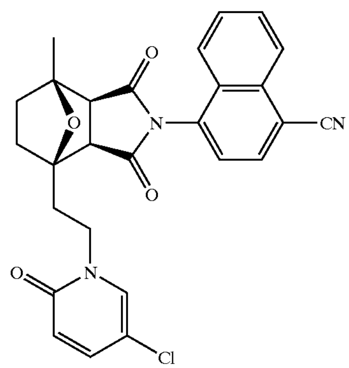
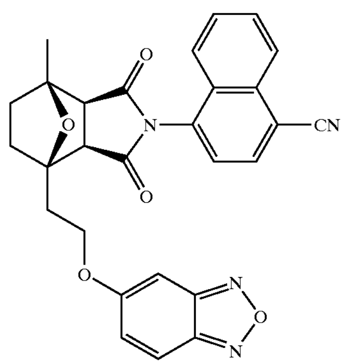
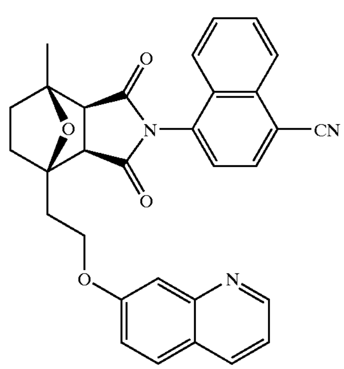
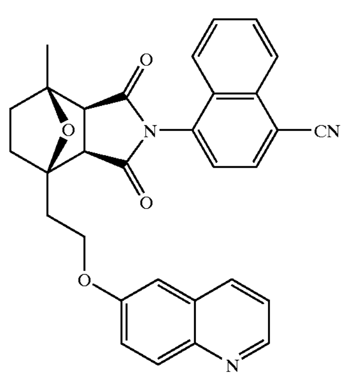
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
646		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(5-Chloro-2-oxo-1(2H)-pyridinyl)ethyl]octahydro-7-methy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.13 LCMS $[M + H]^+$ = 488.1	496
647		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(2,1,3-Benzoxadiazol-5-yloxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.15 LCMS $[M + H]^+$ = 495.2	496
648		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-(7-quinolinyloxy)ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	3.93 LCMS $[M + H]^+$ = 504.2	496
649		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-(6-quinolinyloxy)ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	4.01 LCMS $[M + H]^+$ = 504.2	496

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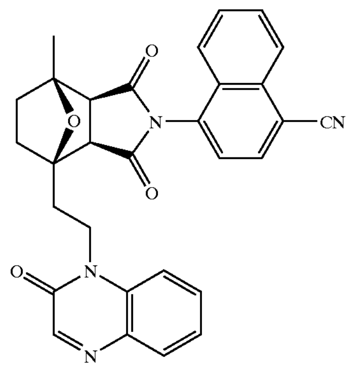
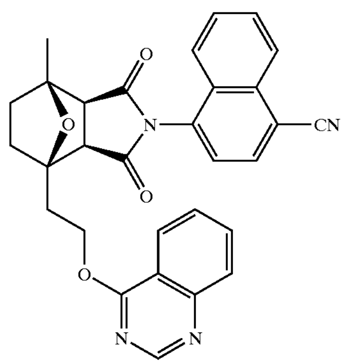
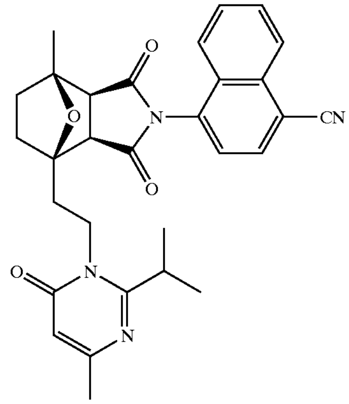
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
650		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-(2-oxo-1(2H)-quinoxalinyl)ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	3.33 LCMS $[M + H]^+$ = 505.2	496
651		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-(4-quinazolinyloxy)ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	3.39 LCMS $[M + H]^+$ = 505.2	496
652		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-[4-methyl-2-(1-methylethyl)-6-oxo-1(6H)-pyrimidinyl]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.72 LCMS $[M + H]^+$ = 511.3	496

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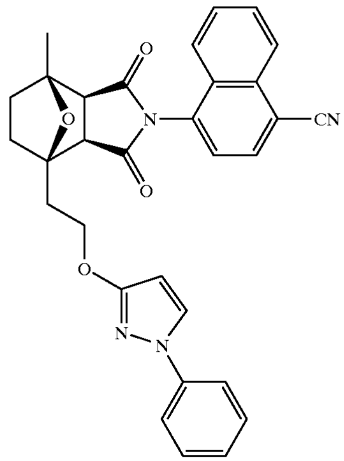
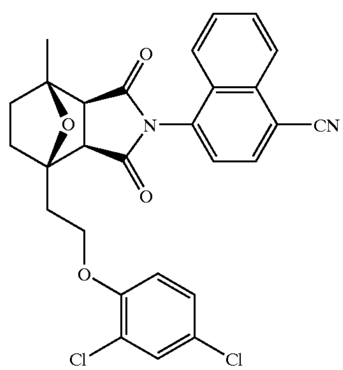
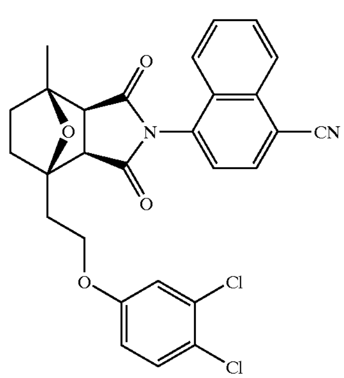
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
653		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[(1-phenyl-1H-pyrazol-3-yl)oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	3.48 LCMS $[M]^+$ = 519.2	496
654		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(2,4-Dichlorophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.85 LCMS $[M + H]^+$ = 521.1	496
655		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(3,4-Dichlorophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.86 LCMS $[M + H]^+$ = 521.1	496

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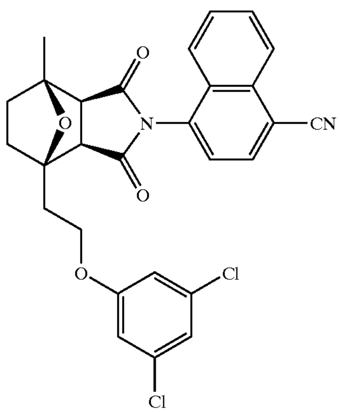
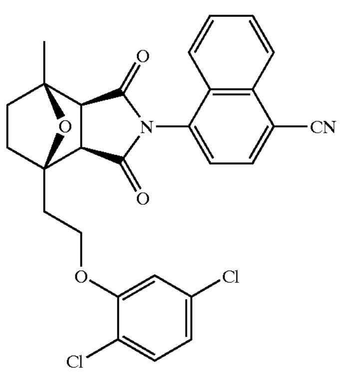
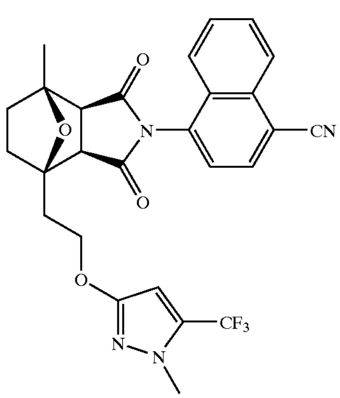
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
656		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(3,5-Dichlorophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.47 LCMS $[M + H]^+$ = 521.1	496
657		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(2,5-Dichlorophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.33 LCMS $[M + H]^+$ = 521.1	496
658		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-[[1-methyl-5-(trifluoromethyl)-1H-pyrazol-3-yl]oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	3.87 LCMS $[M + H]^+$ = 525.2	496

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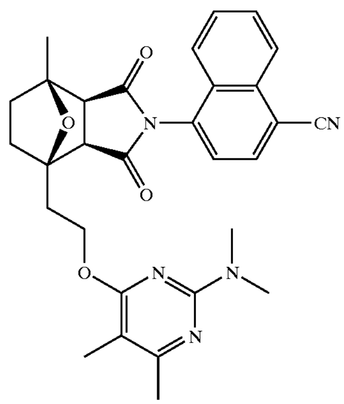
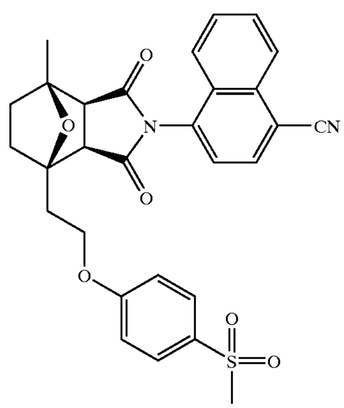
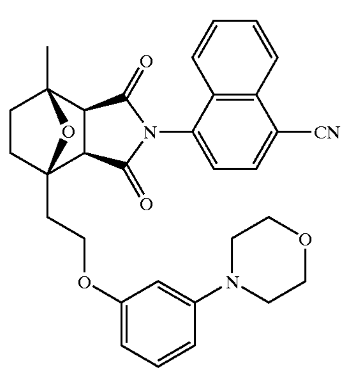
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
659		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[[2-(Dimethylamino)-5,6-dimethyl-4-pyrimidinyl]oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	2.71 LCMS $[M + H]^+$ = 526.2	496
660		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-[4-(methylsulfonyl)phenoxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.91 LCMS $[M + H]^+$ = 531.2	496
661		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-[3-(4-morpholinyl)phenoxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	3.78 LCMS $[M + H]^+$ = 538.2	496

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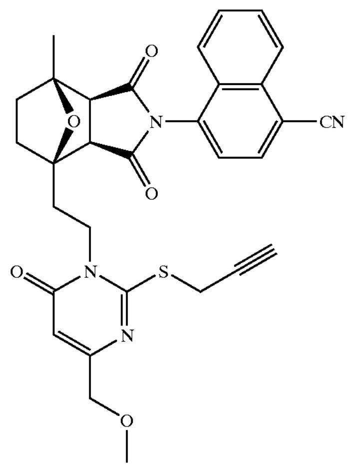
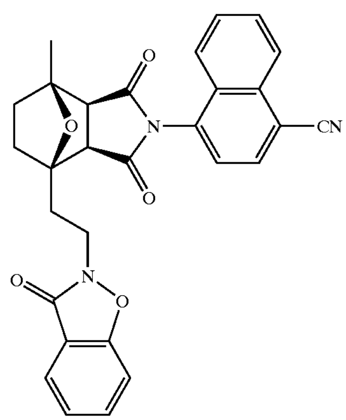
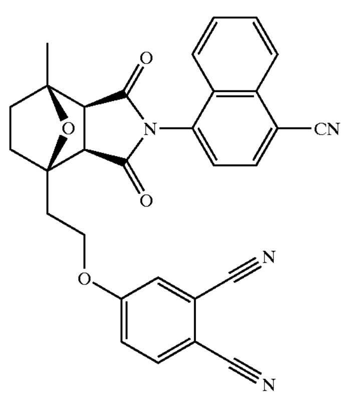
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
662		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-[4-(methoxymethyl)-6-oxo-2-(2-propynylthio)-1(6H)-pyrimidinyl]ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	4.18 LCMS $[M + H]^+$ = 569.2	496
663		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-(3-oxo-1,2-benzisoxazol-2(3H)-yl)ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.18 LCMS $[M + H]^+$ = 494.2	496
664		[3aS-(3aα,4β,7β,7aα)]-4-[2-[2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy]-1,2-benzenedicarbonitrile	4.30 LCMS $[M + H]^+$ = 503.1	496

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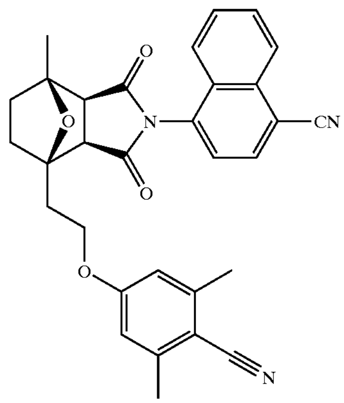
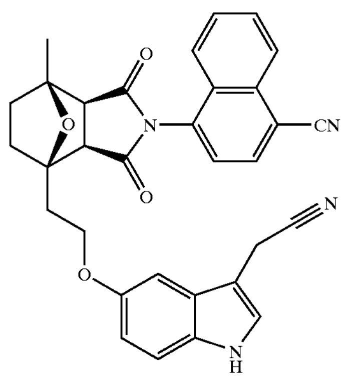
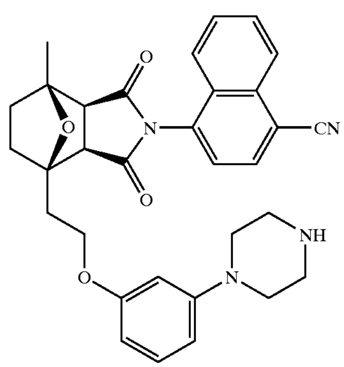
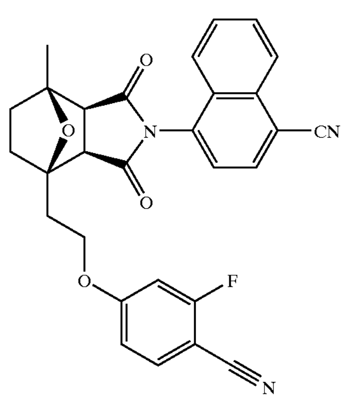
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
665		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(4-Cyano-3,5-dimethylphenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.13 LCMS $[M + H]^+$ = 506.2	496
666		[3aS-(3aα,4β,7β,7aα)]-5-[2-[2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy]-1H-indole-3-acetonitrile, trifluoroacetate (1:1)	3.36 LCMS $[M + H]^+$ = 531.2	496
667		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[3-(1-piperazinyl)phenoxy] ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	3.81 LCMS $[M + H]^+$ = 537.2	496
668		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(4-Cyano-3-fluorophenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.89 LCMS $[M + H]^+$ = 496.2	496

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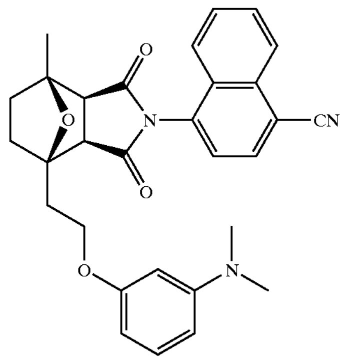
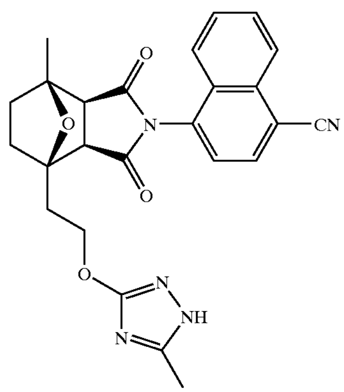
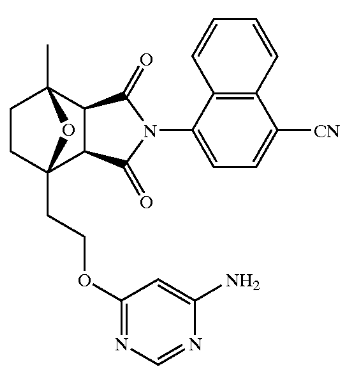
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
669		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[3-(Dimethylamino)phenoxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	3.37 LCMS $[M + H]^+$ = 496.2	496
670		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-[(5-methyl-1H-1,2,4-triazol-3-yl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	3.26 LCMS $[M + H]^+$ = 458.2	496
671		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[(6-Amino-4-pyrimidinyl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	2.97 LCMS $[M + H]^+$ = 470.2	496

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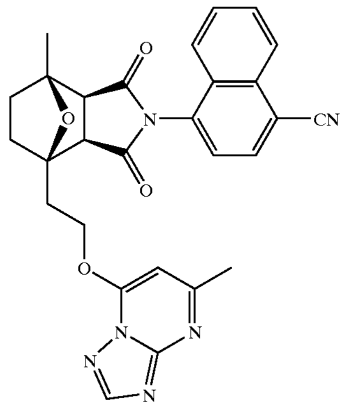
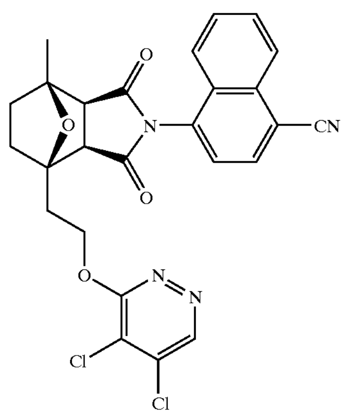
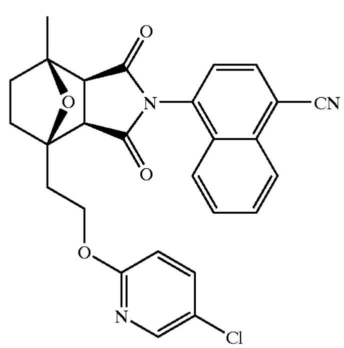
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
672		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-[(5-methyl[1,2,4]triazolo[1,5-a]pyrimidin-7-yl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	3.45 LCMS $[M + H]^+$ = 509.2	496
673		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[(4,5-Dichloro-3-pyridazinyl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.79 LCMS $[M + H]^+$ = 523.1	496
674		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.86 LCMS $[M + H]^+$ = 488.4	496

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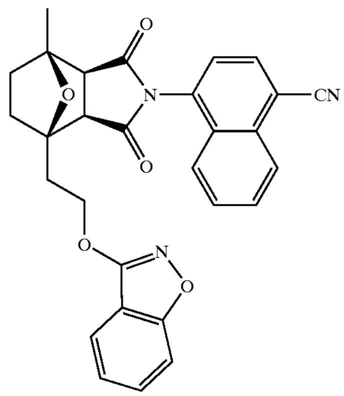
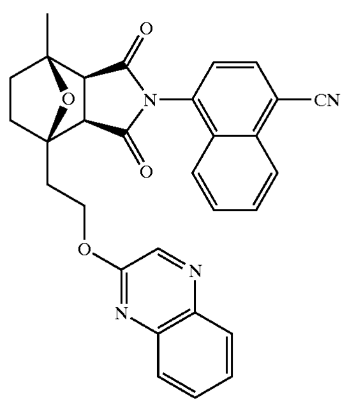
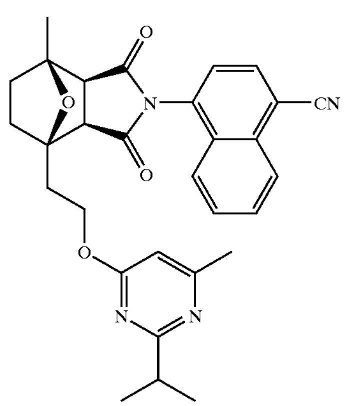
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
675		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(1,2-Benzisoxazol-3-yloxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.78 LCMS $[M + H]^+$ = 494.41	496
676		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-(2-quinoxalinyloxy)ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.83 LCMS $[M + H]^+$ = 505.43	496
677		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-[[6-methyl-2-(1-methylethyl)-4-pyrimidinyl]oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.52 LCMS $[M + H]^+$ = 511.49	496

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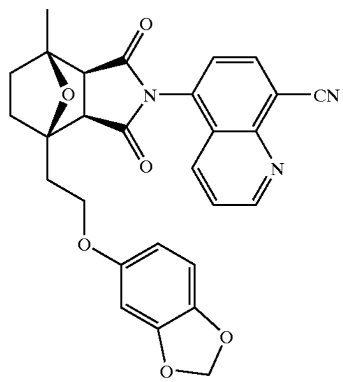
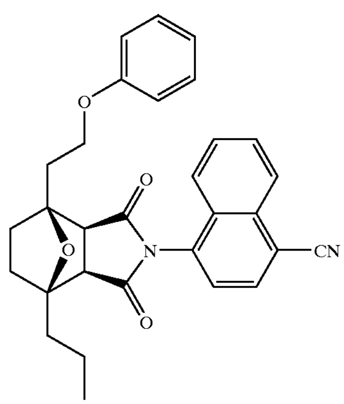
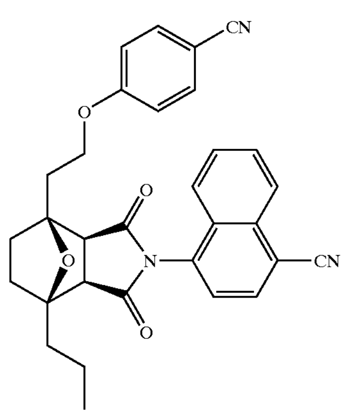
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
678		[3aS-(3aα,4β,7β,7aα)]-5-[4-[2-(1,3-Benzodioxol-5-yloxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.327 LC $[M + H]^+$ = 498.24	467 & 487
679		[3aR-(3aα,4β,7β,7aα)]-4-[Octahydro-1,3-dioxo-4-(2-phenoxyethyl)-7-propyl-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	7.77 & 8.01 atropisomers LC $[M + H]^+$ = 481.4	501
680		[3aR-(3aα,4β,7β,7aα)]-4-[4-[2-(4-Cyanophenoxy)ethyl]octahydro-1,3-dioxo-7-propyl-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	7.42 & 7.68 atropisomers LC $[M + H]^+$ = 506.38	501

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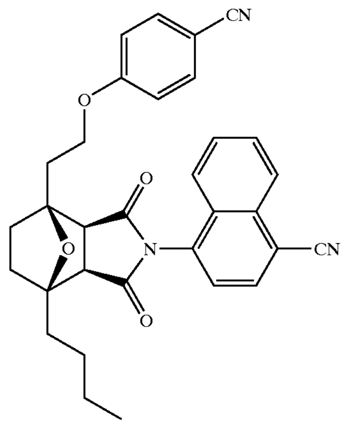
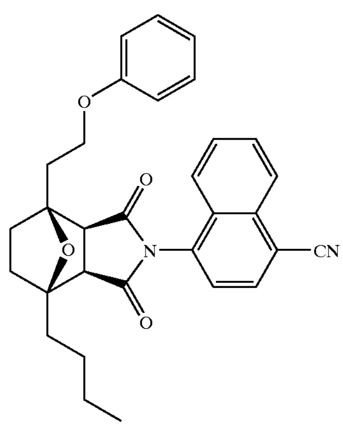
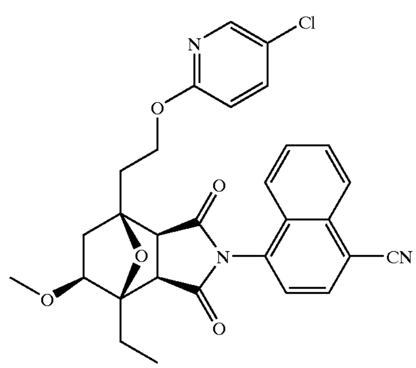
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
681		[3aR-(3aα,4β,7β,7aα)]-4-[4-Butyl-7-[2-(4-cyanophenoxy)ethyl]octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	7.69 & 7.92 atropisomers LC $[M + H]^+$ = 520.38	502
682		[3aR-(3aα,4β,7β,7aα)]-4-[4-Butyloctahydro-1,3-dioxo-7-(2-phenoxyethyl)-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	8.02 & 8.23 atropisomers LC $[M + H]^+$ = 495.33	502
683		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]-4-ethyloctahydro-5-methoxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	7.31 & 7.55 atropisomers LC $[M + H]^+$ = 532.3	504

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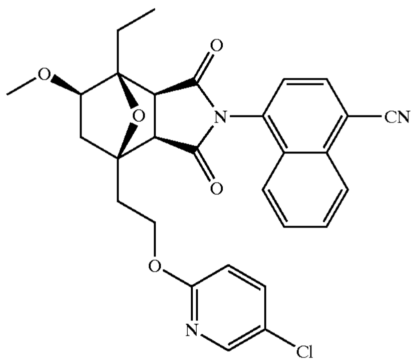
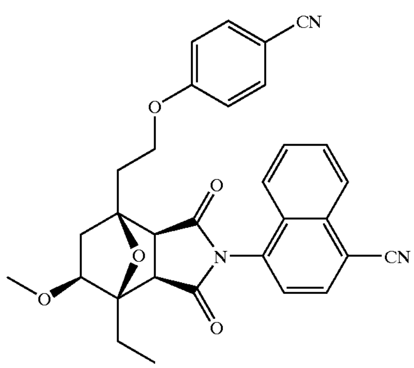
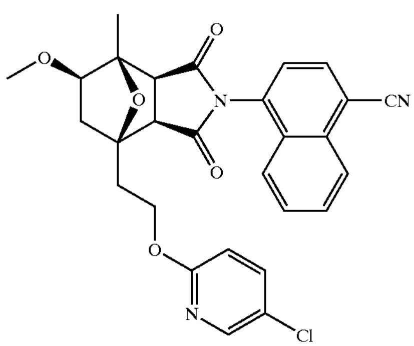
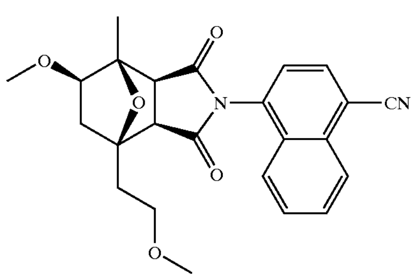
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
684		[3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]-4-ethyloctahydro-5-methoxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	7.31 & 7.55 atropisomers LC $[M + H]^+$ = 532.3	504
685		[3aR-(3aα,4β,5β,7β,7aα)]-4-[7-[2-(4-Cyanophenoxy)ethyl]-4-ethyloctahydro-5-methoxy-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	6.84 & 7.10 atropisomers LC $[M + H]^+$ = 522.33	504
686		[3aS-(3aα,4β,5β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl] octahydro-5-methoxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.37 & 3.52 atropisomers LC $[M + H]^+$ = 518.16	491
687		[3aS-(3aα,4β,5β,7β,7aα)]-4-[Octahydro-5-methoxy-7-(2-methoxyethyl)-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.57 & 2.75 atropisomers LC $[M + H]^+$ = 421.18	491

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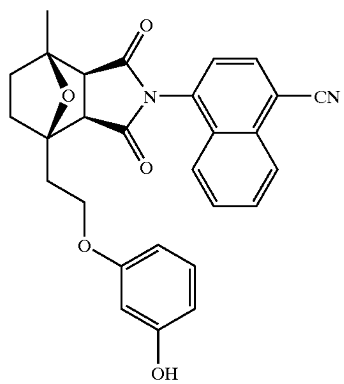
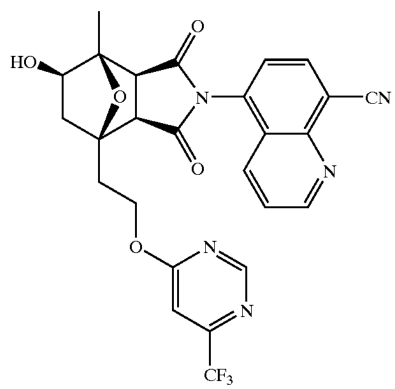
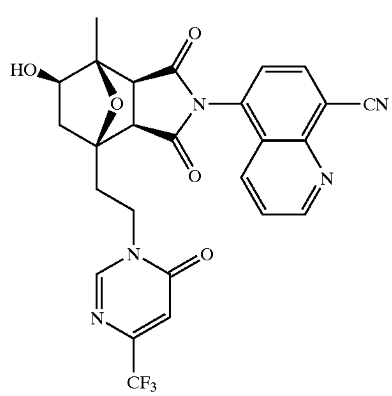
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
688		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-(3-hydroxyphenoxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.38 LCMS $[M + H]^+$ = 469.38	496
689		[3aS-(3aα,4β,5β,7β,7aα)]-5-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[[6-(trifluoromethyl)-4-pyrimidinyl]oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	2.833 LC $[M + H]^+$ = 540.17	485, 486, 487 & 488
690		[3aS-(3aα,4β,5β,7β,7aα)]-5-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[6-oxo-4-(trifluoromethyl)-1(6H)-pyrimidinyl]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	2.59 LC $[M + H]^+$ = 540.16	485, 486, 487 & 488

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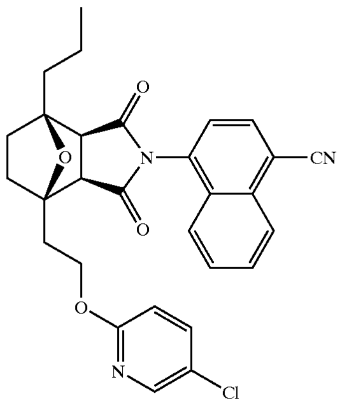
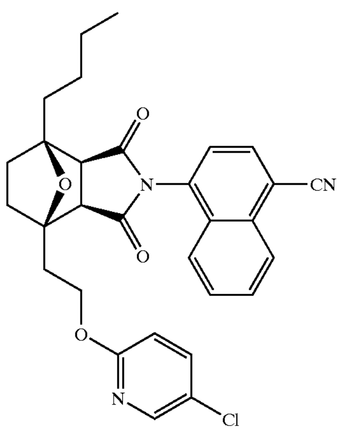
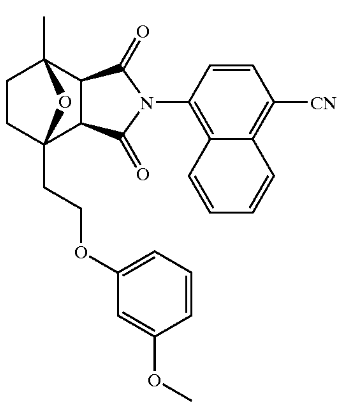
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
691		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-1,3-dioxo-7-propyl-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	7.91 & 8.15 atropisomers LC $[M + H]^+$ = 516.25	501
692		[3aS-(3aα,4β,7β,7aα)]-4-[4-Butyl-7-[2-[(5-chloro-2-pyridinyl)oxy]ethyl]octahydro-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	8.18 & 8.39 atropisomers LC $[M + H]^+$ = 530.28	502
693		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-(3-methoxyphenoxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.97 LCMS $[M + H]^+$ = 483.1	496

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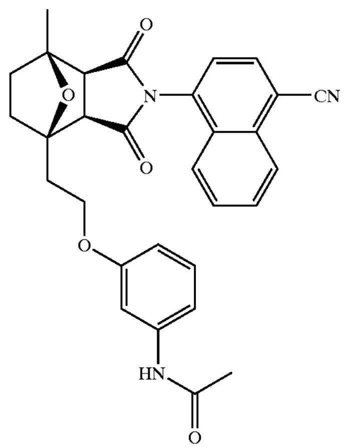
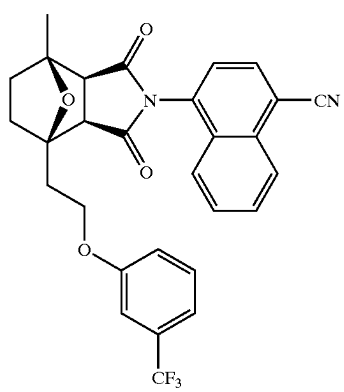
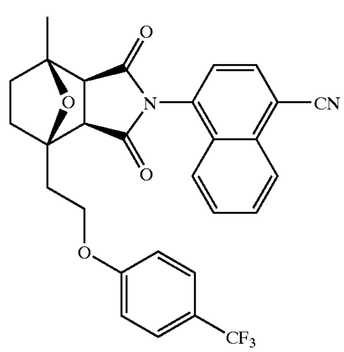
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
694		N-[3-[2-[3aS-(3aα,4β,7β,7aα)]-2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy]phenylacetamide	3.42 LCMS $[M + H]^+$ = 510.1	496
695		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[3-(trifluoromethyl)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.83 LCMS $[M + H]^+$ = 521.1	496
696		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[4-(trifluoromethyl)phenoxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.86 LCMS $[M + H]^+$ = 521.2	496

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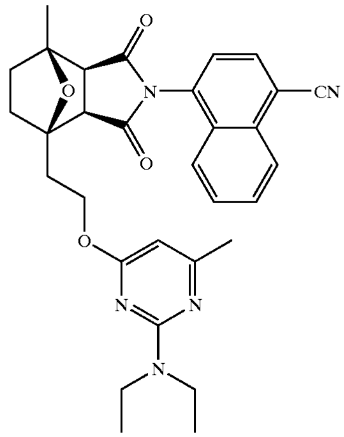
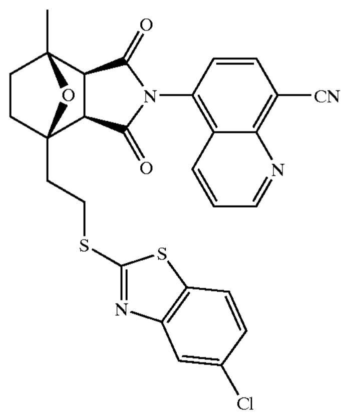
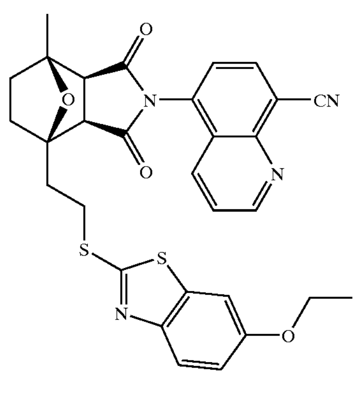
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
697		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[[2-(Diethylamino)-6-methyl-4-pyrimidinyl[oxy]ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.63 LCMS $[M + H]^+$ = 540.2	496
698		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[(5-Chloro-2-benzothiazolyl)thio]ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.15 LCMS $[M]^+$ = 560.0	496
699		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[(6-Ethoxy-2-benzothiazolyl)thio] ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.15 LCMS $[M + H]^+$ = 570.1	496

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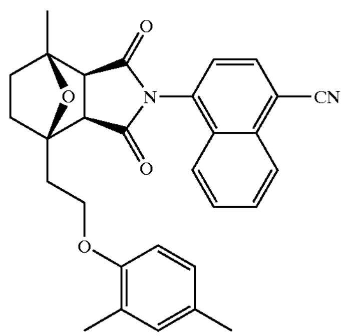
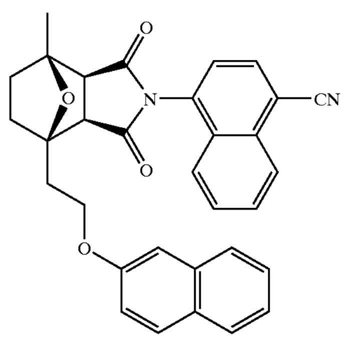
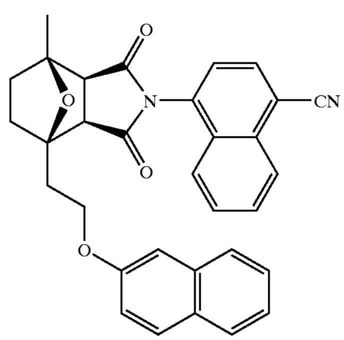
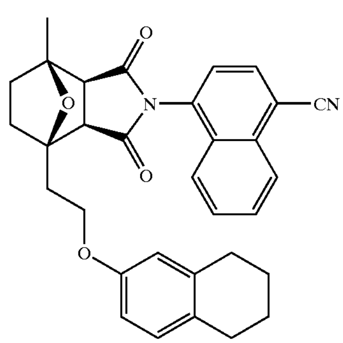
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
700		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(2,4-Dimethylphenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.71 LCMS $[M + H]^+$ = 481.2	496
701		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(2,5-Dimethylphenoxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.79 LCMS $[M + H]^+$ = 481.2	496
702		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-(2-naphthalenyloxy)ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.66 LCMS $[M + H]^+$ = 503.2	496
703		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[(5,6,7,8-tetrahydro-2-naphthalenyl)oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.72 LCMS $[M + H]^+$ = 507.2	496

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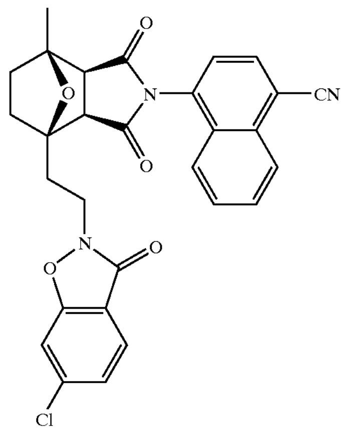
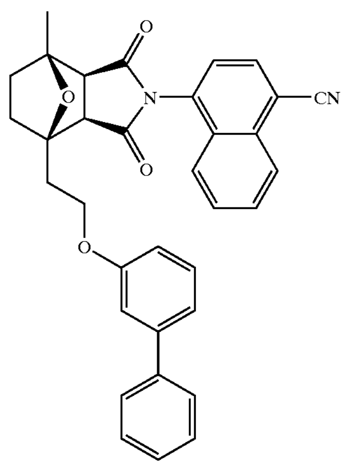
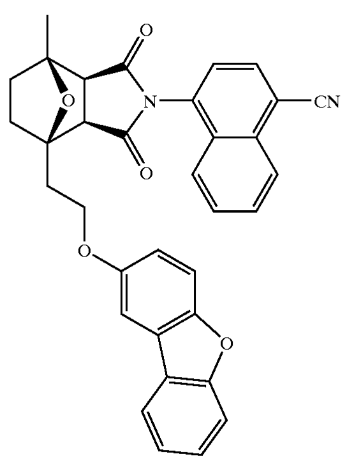
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
704		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(6-Chloro-3-oxo-1,2-benzisoxazol-2(3H)-yl)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.71 LCMS $[M + H]^+$ = 528.1	496
705		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-([1,1'-Biphenyl]-3-yloxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.03 LCMS $[M + H]^+$ = 529.2	496
706		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(2-Dibenzofuranyloxy)ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.51 LCMS $[M + H]^+$ = 543.2	496

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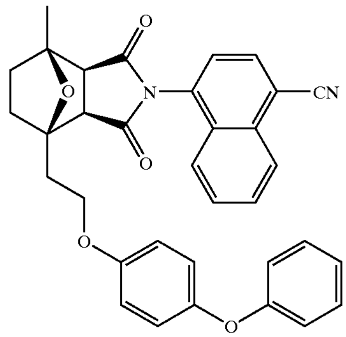
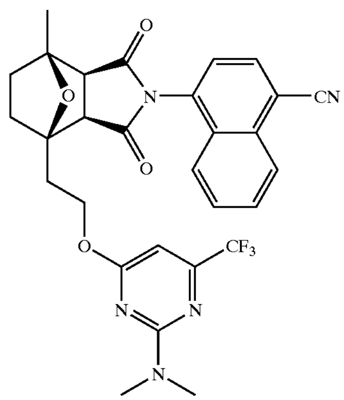
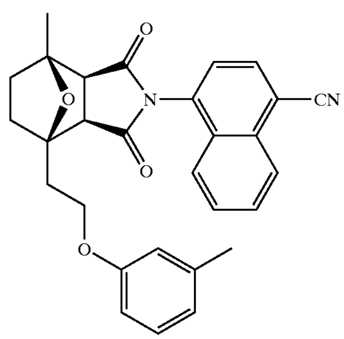
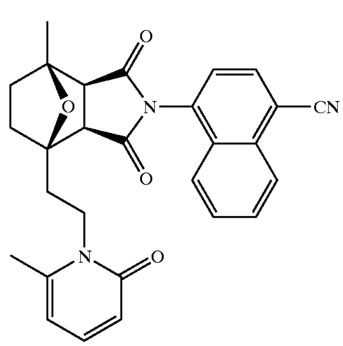
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
707		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-(4-phenoxyphenoxy)ethyl]-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.73 LCMS $[M + H]^+$ = 545.2	496
708		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[[2-(Dimethylamino)-6-(trifluoromethyl)-4-pyrimidinyl]oxy]ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	4.15 LCMS $[M + H]^+$ = 566.2	496
709		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-(3-methylphenoxy)ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.17 LCMS $[M + H]^+$ = 466.5	496
710		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-(6-methyl-2-oxo-1(2H)-pyridinyl)ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.41 LCMS $[M + H]^+$ = 468.2	496

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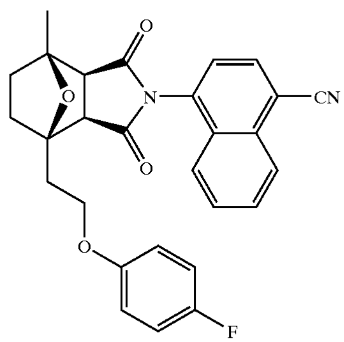
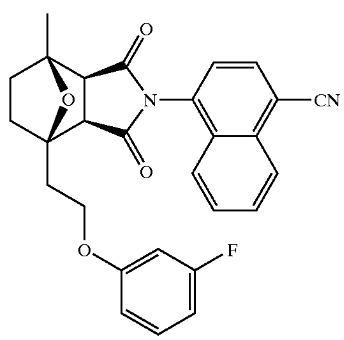
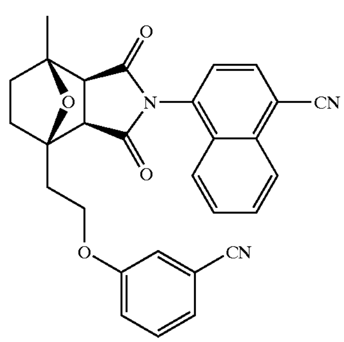
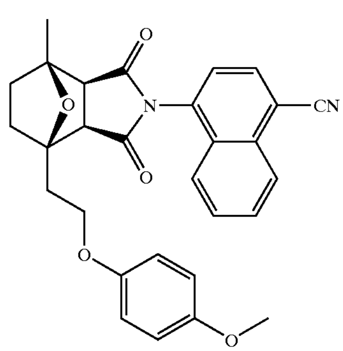
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
711		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(4-Fluorophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.10 LCMS $[M + H]^+$ = 471.2	496
712		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(3-Fluorophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.56 LCMS $[M + H]^+$ = 471.2	496
713		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(3-Cyanophenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.28 LCMS $[M + H]^+$ = 478.2	496
714		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-(4-methoxyphenoxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.58 LCMS $[M + H]^+$ = 483.2	496

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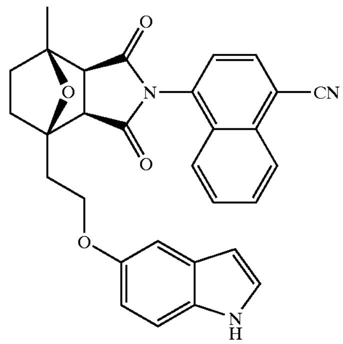
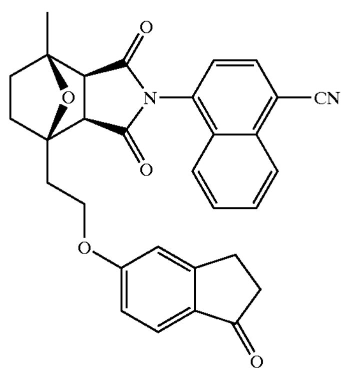
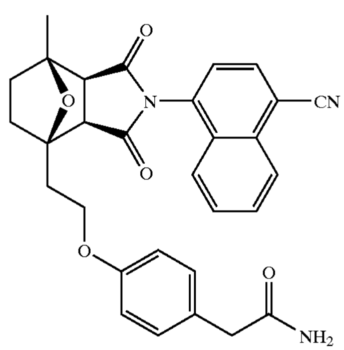
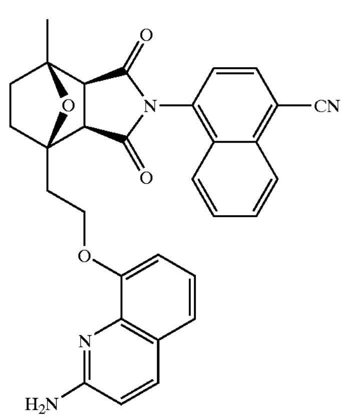
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
715		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-(1H-indol-5-yloxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.40 LCMS $[M + H]^+$ = 492.2	496
716		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[(2,3-Dihydro-1-oxo-1H-inden-5-yl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.20 LCMS $[M + H]^+$ = 507.2	496
717		[3aS-(3aα,4β,7β,7aα)]-4-[2-[2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy] benzeneacetamide	3.26 LCMS $[M + H]^+$ = 510.2	496
718		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[(2-Amino-8-quinolinyl)oxy]ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	2.77 LCMS $[M + H]^+$ = 519.2	496

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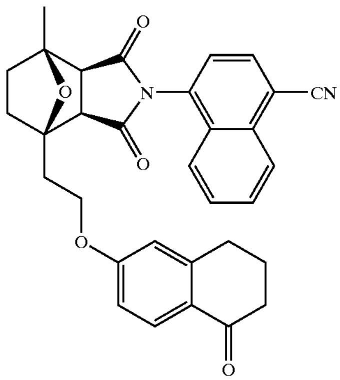
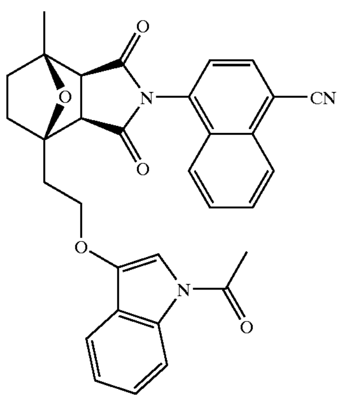
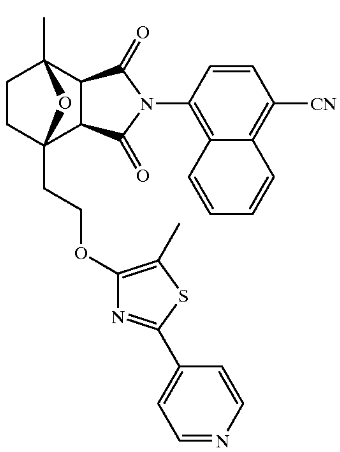
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
719		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-1,3-dioxo-7-[2-[(5,6,7,8-tetrahydro-5-oxo-2-naphthalenyl)oxy]ethyl] 4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.88 LCMS $[M + H]^+$ = 521.2	496
720		[3aS-(3aα,4β,7β,7aα)]-1-Acetyl-3-[2-[2-(4-cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy]-1H-indole	4.03 LCMS $[M + H]^+$ = 534.2	496
721		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-[[5-methyl-2(4-pyridinyl)-4-thiazolyl]oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile, trifluoroacetate (1:1)	4.02 LCMS $[M + H]^+$ = 551.2	496

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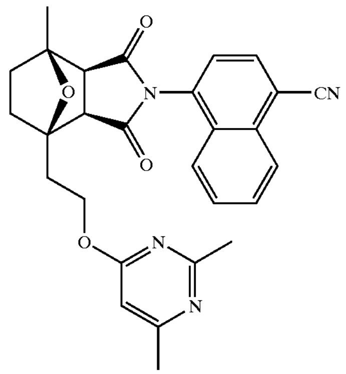
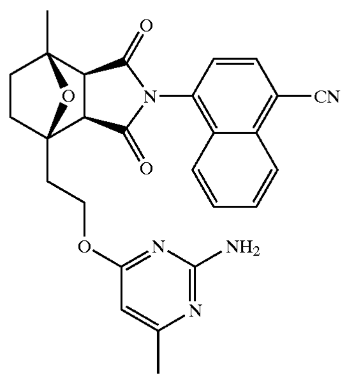
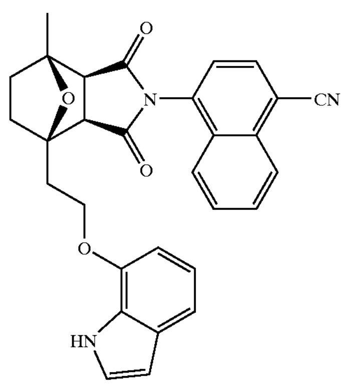
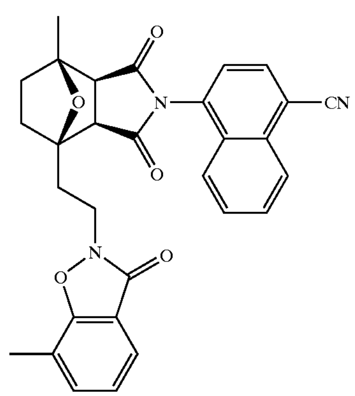
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
722		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[(2,6-Dimethyl-4-pyrimidinyl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.74 LCMS $[M + H]^+$ = 483.2	496
723		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[(2-Amino-6-methyl-4-pyrimidinyl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.71 LCMS $[M + H]^+$ = 484.2	496
724		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-(1H-indol-7-yloxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.23 LCMS $[M + H]^+$ = 492.2	496
725		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-(7-methyl-3-oxo-1,2-benzisoxazol-2(3H)-yl)ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.02 LCMS $[M + H]^+$ = 508.2	496

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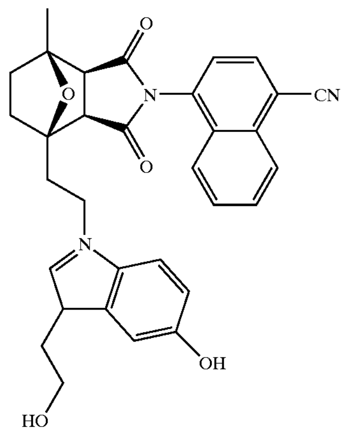
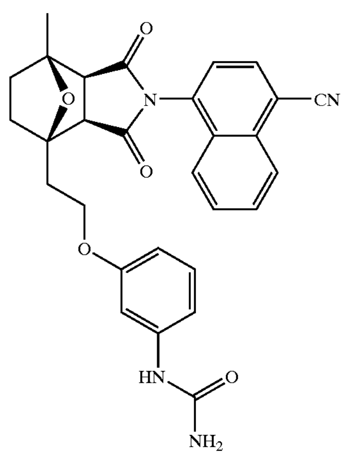
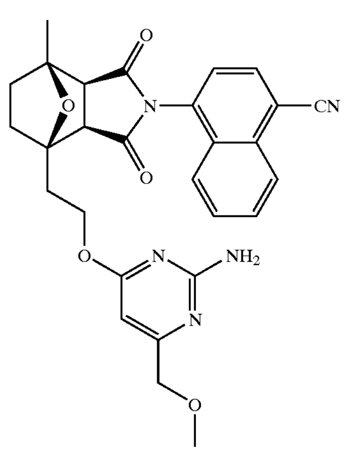
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
726		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-[5-hydroxy-3-(2-hydroxyethyl)-1H-indol-1-yl]ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	2.94 LCMS $[M + H]^+$ = 536.2	496
727		N-[3-[2-[[3aS-(3aα,4β,7β,7aα)]-2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy]phenyl]urea	4.26 LCMS $[M + H]^+$ = 511.2	496
728		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[[2-Amino-6-(methoxymethyl)-4-pyrimidinyl]oxy]ethyl] octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.18 LCMS $[M + H]^+$ = 514.2	496

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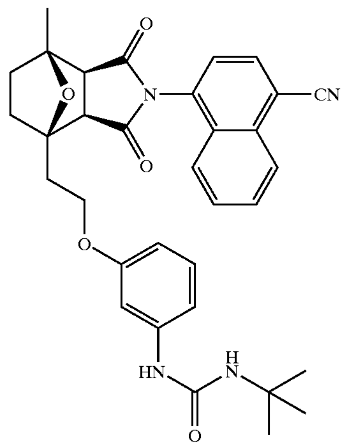
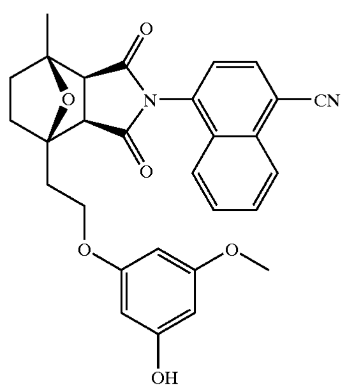
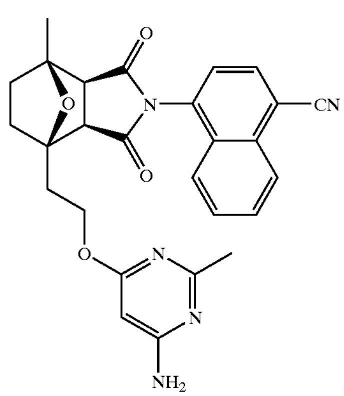
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
729		[N-[3-[2-[[3aS-(3aα,4β,7β,7aα)]-2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy]phenyl]-N'-(1-1-dimethylethyl)urea	3.13 LCMS $[M + H]^+$ = 567.2	496
730		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-[2-(3-hydroxy-5-methoxyphenoxy)ethyl]-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.22 LCMS $[M + H]^+$ = 499.2	496
731		[3aS-(3aα,4β,7β7aα)]-4-[4-[2-[(6-Amino-2-methyl-4-pyrimidinyl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.19 LCMS $[M + H]^+$ = 484.2	496

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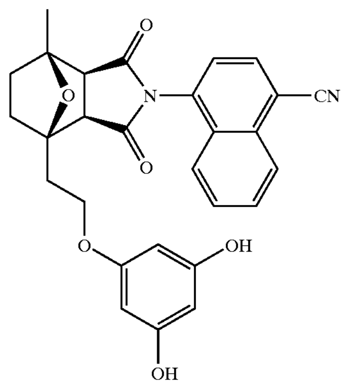
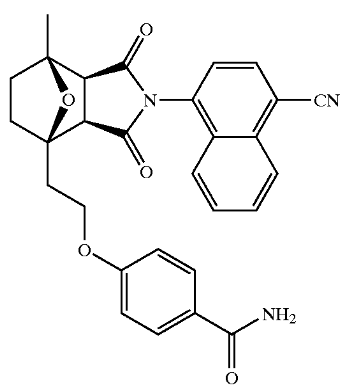
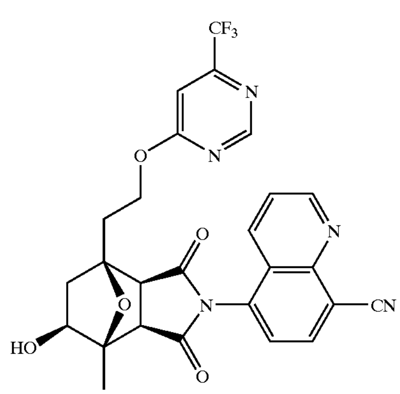
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
732		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-(3,5-Dihydroxyphenoxy)ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.73 LCMS $[M + H]^+$ = 485.1	496
733		[3aS-(3aα,4β,7β,7aα)]-4-[2-[2-(4-Cyano-1-naphthalenyl)octahydro-7-methyl-1,3-dioxo-4,7-epoxy-4H-isoindol-4-yl]ethoxy]benzamide	4.11 LCMS $[M + H]^+$ = 496.2	496
734		[3aR-(3aα,4β,5β,7β,7aα)]-5-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[[6-(trifluoromethyl)-4-pyrimidinyl]oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	2.847 LC $[M + H]^+$ = 540.14	485, 486, 487 & 488

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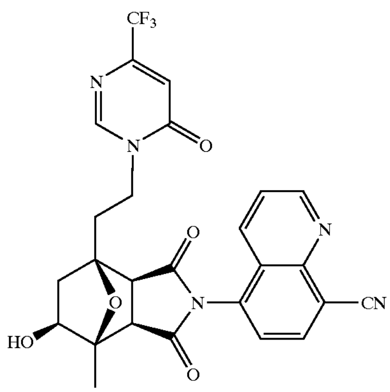
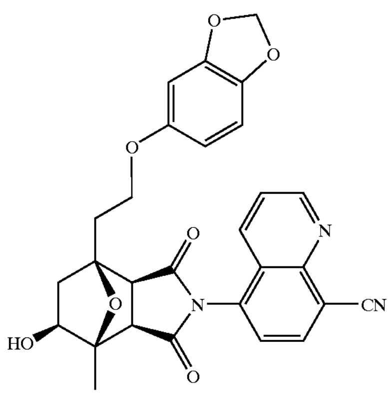
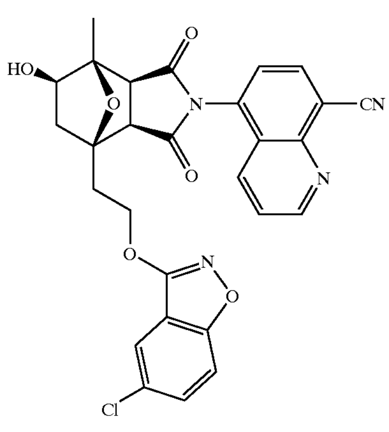
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
735		[3aR-(3aα,4β,5β,7β,7aα)]-5-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[6-oxo-4-(trifluoromethyl)-1(6H)-pyrimidinyl]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	2.577 LCMS $[M+H]^+$ = 540.14	485, 486, 487 & 488
736		[3aR-(3aα,4β,5β,7β,7aα)]-5-[7-[2-(1,3-Benzodioxol-5-yloxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	2.867 LCMS $[M+H]^+$ = 514.16	485 486, 487 & 488
737		[3aS-(3aα,4β,5β,7β,7aα)]-5-[7-[2-[(5-Chloro-1,2-benzisoxazol-3-yl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.24 LCMS $[M+H]^+$ = 545.12	485, 486, 487 & 488

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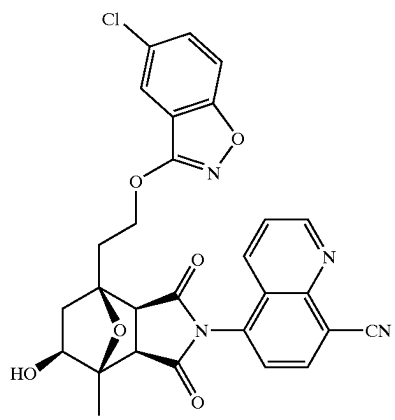
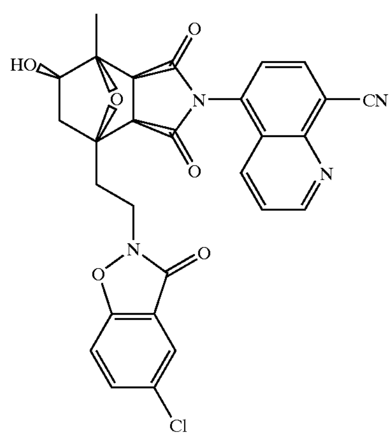
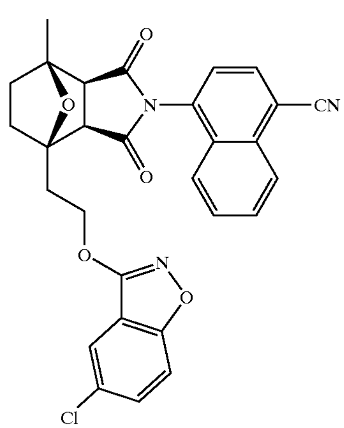
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
738		[3aR-(3aα,4β,5β,7β,7aα)]-5-[7-[2-[(5-Chloro-1,2-benzisoxazol-3-yl)oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.25 LCMS $[M + H]^+$ = 545.12	485, 486, 487 & 488
739		[3aS-(3aα,4β,5β,7β,7aα)]-5-[7-[2-(5-Chloro-3-oxo-1,2-benzisoxazol-2(3H)-yl)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	2.863 LCMS $[M + H]^+$ = 545.12	485, 486, 487 & 488
740		[3aS-(3aα,4β,7β,7aα)]-4-[4-[2-[(6-Chloro-1,2-benzisoxazol-3-yl)oxy]ethyl]octahydro-7-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	4.04 LCMS $[M + H]^+$ = 528.1	496

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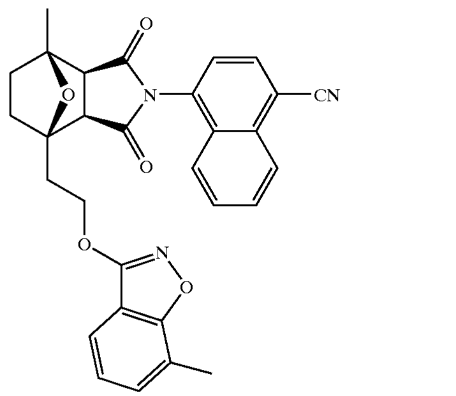
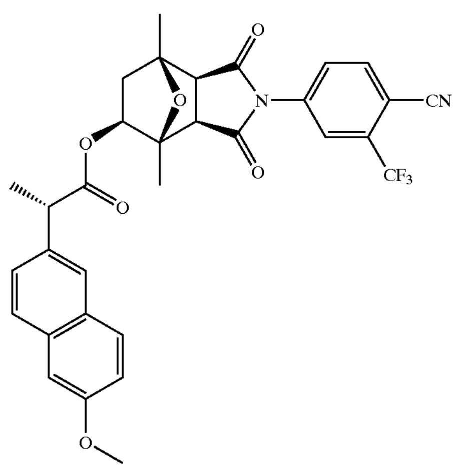
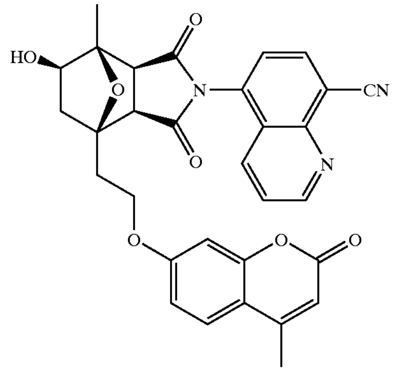
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
741		[3aS-(3aα,4β,7β,7aα)]-4-[Octahydro-4-methyl-7-[2-[(7-methyl-1,2-benzisoxazol-3-yl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile	3.94 LCMS $[M + H]^+$ = 508.1	496
742		[(αS)-6-Methoxy-α-methyl-2-naphthaleneacetic acid, [3aR-(3aα,4β,5β,7β,7aα)]-2-[4-cyano-3-(trifluoromethyl)phenyl]octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-1H-isoindol-5-yl ester	$[M - H]^-$ = 591.3	483
743		[3aS-(3aα,4β,5β,7β,7aα)]-5-[Octahydro-5-hydroxy-4-methyl-7-[2-[(4-methyl-2-oxo-2H-1-benzopyran-7-yl)oxy]ethyl]-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	2.89 LCMS $[M + H]^+$ = 552.19	485, 486, 487 & 488

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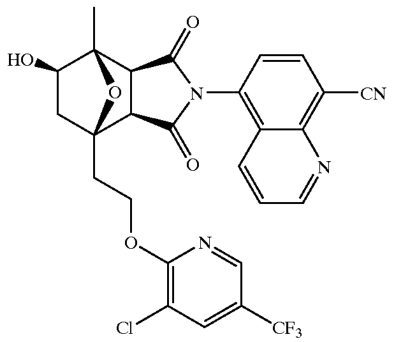
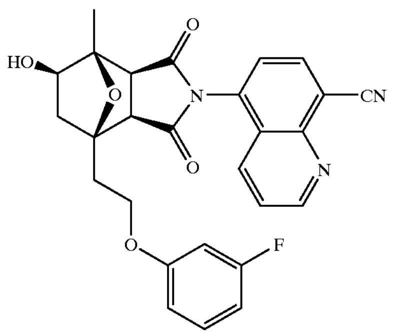
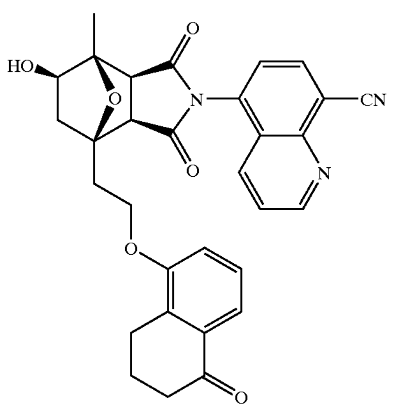
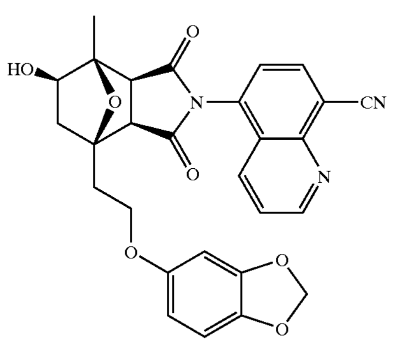
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
744		[3aS-(3aα,4β,5β,7β,7aα)]-5-[7-[2-[[3-Chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.46 LCMS $[M + H]^+$ = 573.12	485, 486, 487 & 488
745		[3aS-(3aα,4β,5β,7β,7aα)]-5-[7-[2-(3-Fluorophenoxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.05 LCMS $[M + H]^+$ = 488.03	485, 486, 487 & 488
746		[3aS-(3aα,4β,5β,7β,7aα)]-5-[Octahydro-5-hydroxy-4-methyl-1,3-dioxo-7-[2-[(5,6,7,8-tetrahydro-5-oxo-1-naphthalenyl)oxy]ethyl]-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	2.98 LCMS $[M + H]^+$ = 538.23	485, 486, 487 & 488
751		[3aS-(3aα,4β,5β,7β,7aα)]-5-[7-[2-(1,3-Benzodioxol-5-yloxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	2.86 LCMS $[M + H]^+$ = 514.16	485, 486, 487 & 488

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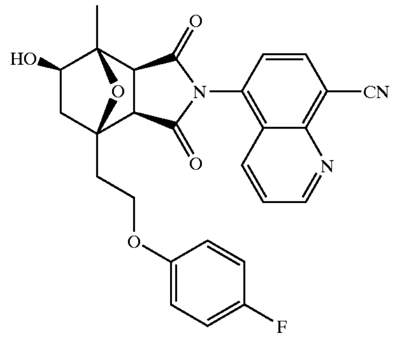
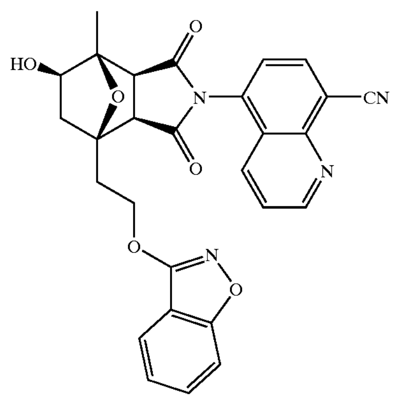
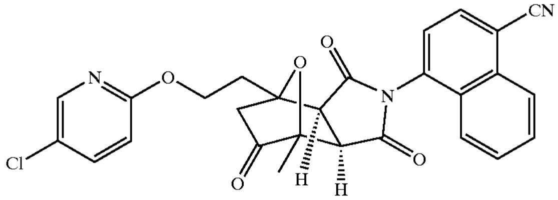
Ex. No.	Compound Structure	Compound Name	Retention Time Min./ Molecular Mass	Pro. of Ex.
752		[3aS-(3aα,4β,5β,7β,7aα)]-5-[7-[2-(4-Fluorophenoxy)ethyl] octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	3.02 LCMS $[M + H]^+$ = 488.2	485, 486, 487 & 488
753		[3aS-(3aα,4β,5β,7β,7aα)]-5-[7-[2-(1,2-Benzisoxazol-3-yloxy)ethyl]octahydro-5-hydroxy-4-methyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-8-quinolinecarbonitrile	2.94 LCMS $[M + H]^+$ = 511.2	485, 486, 487 & 488

EXAMPLE 747

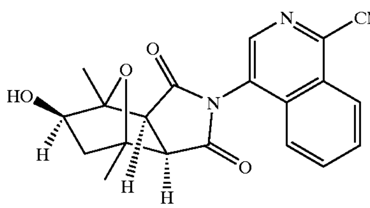
[3aR-(3aα,4β,7β,7aα)]-4-[7-[2-[(5-Chloro-2-pyridinyl)oxy]ethyl]octahydro-4-methyl-1,3,5-trioxo-4,7-epoxy-2H-isoindol-2-yl]-1-naphthalenecarbonitrile (747)



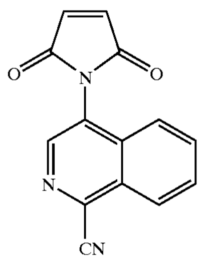
Dess-Martin periodinane (122 mg, 0.29 mmol, prepared as described in Ishiharaa, J., T. Fukuzakia, et al. *Tetrahedron Letters* 40(10), 1907–1910 (1999)) was added to a solution of the compound 490A (120 mg, 0.24 mmol) in dichloromethane (2.5 mL) under nitrogen and the mixture was stirred for 4 h. The reaction was half concentrated under a stream of nitrogen and was applied on a flash cartridge (Jones 2 g) with celite on top and was eluted with chloroform:heptane (9:1) to chloroform:acetone (4:1) to give 148 mg of a still impure white solid. The solid was disolved in dichloromethane (5 mL) and heptane (3 mL) and the precipitate was filtered over 1 g silica and was eluted with dichloromethane to chloroform:acetone (4:1). Fractions 3–9 (58.8 mg white solid) was almost pure and fractions 10–13 (68 mg white foam) were still impure. Fractions 3–9 were purified over silica (1 g) using heptane to heptane:ethyl acetate (1:1) as eluant to give 35.8 mg (30% yield) compound 747 as a white solid. Fractions 10–13 were purified by adding ~400 mg of silica to an excess solution of crude compound 747 in ethyl acetate and heptane and concentrating it. The silica was then put on top of a preconditioned (heptane) silica column (1 g) and was eluted with a gradient from heptane to heptane ethyl acetate (1:1) to give an additional 36.7 mg (31% yield) of compound 747 as a white solid. HPLC: 94% at 3.46 & 3.59 min (atropisomers, retention time) (YMC S5 ODS 4.6×50 mm, 4 mL min, 4 min gradient 100% A to 100% B (A: 10% methanol, 89.1% water, 0.1% TFA; B: 10% water, 89.1% methanol, 0.1% TFA, 4 mL/min, monitoring at 220 nm). MS (ES): m/z 502.11 $[M+H]^+$.

EXAMPLE 748

(3aα,4β,5β,7β,7aα)-4-[Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-isoquinolinecarbonitrile (748D)

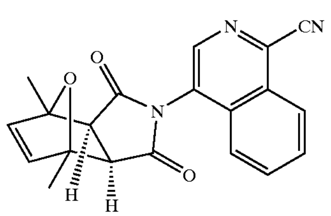


A. 4-(2,5-Dioxo-2,5-dihydro-pyrrol-1-yl)-isoquinoline-1-carbonitrile (748A)



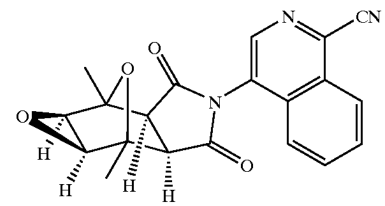
A mixture of compound 470D (200 mg, 1.18 mmol) and maleic anhydride (470 mg, 4.7 mmol) in glacial acetic acid (5 mL) was heated to reflux for 4 hours. After removing the volatiles in vacuo, the residue was partitioned between ethyl acetate (50 mL) and water (50 mL). The organic layer was washed with saturated sodium bicarbonate solution (2×50 mL) and brine (50 mL). After drying over magnesium sulfate, the organic layer was filtered through a 1×5 cm plug of silica gel. The filtrate was concentrated to afford 263 mg (90%) of 748a as an off-white solid. HPLC: 99% at 1.12 nin (Phenomenex 5 micron ODS 4.6×30 mm, 10%–90% aqueous methanol over 2 minute gradient with 0.1% TFA, detecting at 254 nm). MS (ES): m/Z 250.2 $[M+H]^+$.

B. (3aα,4β,7β,7aα)-4-(1,3,3a,4,7,7a-Hexahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl)-1-isoquinolinecarbonitrile (748B)



A mixture of compound 748A (250 mg, 1 mmol) and 2,5-dimethylfuran (4 mL) was heated to 60° C. for 2 h. At 15 min, the reaction mixture became homogeneous. A precipitate formed at 45 minutes. After cooling to 25° C., the reaction mixture was diluted with hexane and the filter cake was washed with ethyl ether:hexane, 1:1. Drying under high vacuum afforded 270 mg (78%) of compound 748B as a light yellow solid. ^{1}HNMR-400 MHz ($CDCl_3$): δ8.55 (s, 1H), 8.54 (m, 1H), 7.86 (m, 3H), 6.45 (s, 2H), 3.18 (s, 2H), 1.81 (s, 6H).

C. (1aα,2β,2aα,5aα,6β,6aα)-4-[Octahydro-2,6-dimethyl-3,5-dioxo-2,6-epoxy-4H-oxireno[f]isoindol-4-yl]-1-isoquinolinecarbonitrile (748C)



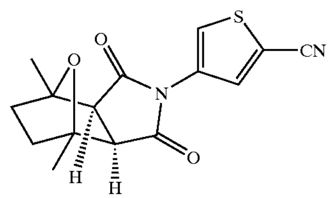
m-CPBA, (70%, 110 mg, 0.45 mmol) was added to a solution of compound 748B (100 mg, 0.29 mmol) in 3 mL of dichloromethane at 25° C. After 1 h, additional m-CPBA, (70%, 110 mg, 0.45 mmol) was added and the reaction mixture was stirred an additional 18 h. After partitioning the reaction mixture between ethyl acetate (30 mL) and water (30 mL), the organic layer was washed with saturated sodium bisulfite solution (30 mL), saturated sodium bicarbonate solution (2×30 mL) and brine (30 mL). The sample was dried over magnesium sulfate and concentration to yield 103 mg (98%) of compound 748C as an off-white solid. HPLC: 99% at 1.09 & 1.22 min (atropisomers, retention time) (Phenomenex 5 micron ODS 4.6×30 mm, 10%–90% aqueous methanol over a 2 min gradient with 0.1% TFA, detecting at 254 nm). MS (ES): m/z 362.07 $[M+H]^+$.

D. (3aα,4β,5β,7β,7aα)-4-[Octahydro-5-hydroxy-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-1-isoquinolinecarbonitrile (748D)

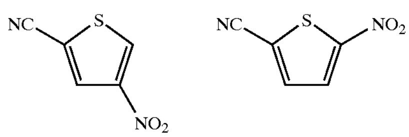
A 0.5 M solution of bis(cyclopentadienyl)titanium chloride in THF (1.2 mL, 0.6 mmol) was added dropwise over 20 min to a well stirred suspension of compound 748C (103 mg, 0.29 mmol) in THF (2.5 mL) and 1,4-cyclohexadiene (1.2 mL) at 60° C. After stirring 1 h at 60° C., the reaction mixture was partitioned between 1N HCl (40 mL) and ethyl acetate (50 mL). The pH of the aqueous layer was adjusted to 7 with solid sodium bicarbonate. After extracting the aqueous with ethyl acetate, the combined organic layers were dried over sodium sulfate. Decolorizing carbon (~1 g) was added and the mixture was allowed to stand for 18 h. Filtration and concentration of the filtrate in vacuo afforded a yellow oil that was chromatographed on a 2.5×15 cm silica gel column, using dichloro-methane:acetone, 6:4 as the mobile phase. Concentration of the product containing fractions in vacuo gave a partially purified residue that was subjected to preparative thin layer silica gel chromatography, using dichloromethane:acetone, 6:4 as the mobile phase. Extraction of the desired band with CH_2Cl_2, filtration and concentration of the filtrate in vacuo yielded 3 mg (31%) of compound 748D as an off-white solid. HPLC conditions: 95% at 1.46 min (Phenomenex 5 micron ODS 4.6×30 mm, 10%–90% aqueous methanol over 2 minute gradient with 0.1% TFA, detecting at 254 nm). MS (ES): m/z 364.19 $[M+H]^+$.

EXAMPLE 749

(3aα,4β,7β,7aα)-4-[Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-thiophenecarbonitrile (749C)

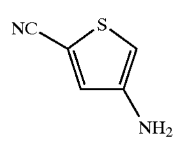


A. 2-Cyano-4-nitrothiophene (749Ai) & 2-Cyano-5-nitrothiophene (749Aii)



Fuming nitric acid (21 mL) was slowly added to glacial acetic acid (105 mL) and the mixture was then cooled in an ice-bath. 2-Cyanothiophene (7.98 g, 73.1 mmol) was dissolved in 20 mL of acetic anhydride and added dropwise to the above acid mixture such that the temperature did not exceed 25° C. Upon completion of the addition, the reaction mixture was warmed to 22° C. and stirred for 48 h. The reaction was poured over 400 mL of ice and extracted with 200 mL of diethylether. The ether layer was isolated, washed with water, followed by brine and dried over $MgSO_4$. Filtration and concentration in vacuo gave a sticky, orange residue which was purified by column chromatography using 1:1 hexanes/methylene chloride as the eluent to give 1.69 g (15%) of compound 749Ai as a white solid and 1.71 g (15%) of compound 749Aii as a yellow crystalline substance. Compound 749Ai: HPLC: 0.73 minutes (retention time) (Phenomenex column 30×4.6 mm eluting with 10–90% aqueous methanol over 2 minutes containing 0.1% TFA, 5 mL/min, monitoring at 220 nm). Compound 749Aii: HPLC: 99% at 0.89 minutes (retention time) (Phenomenex column 30×4.6 mm eluting with 10–90% aqueous methanol over 2 minutes containing 0.1% TFA, 5 mL/min, monitoring at 220 nm).

B. 4-Amino-2-cyanothiophene (749B)



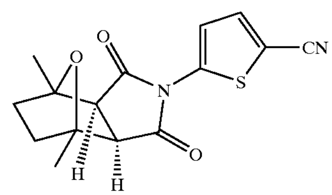
To a 100 mL 3-necked flask was added compound 749Ai (1.42 g, 9.21 mmol) dissolved in ethyl acetate (20 mL) followed by a 10% acetic acid solution (20 mL). The biphasic mixture was heated to 65° C. and then iron powder (2.95 g, 52.9 mmol) was added portion-wise over 5 minutes. After stirring for 1.5 h at 65° C., the reaction was filtered through a bed of Celite and the pad was washed with ethyl acetate. The organic layer was separated, washed with water (3×20 mL), brine and dried over $MgSO_4$. Filtration and concentration in vacuo gave a dark amber residue which was purified by column chromatography using 30% diethyl ether/methylene chloride as the eluent to give 84 mg (73%) of compound 749B as a brown solid. HPLC: 93.4% at 0.26 minutes (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

C. (3aα,4β,7β,7aα)-4-[Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-thiophenecarbonitrile (749C)

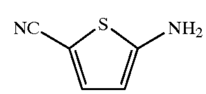
To a Pyrex tube was added compound 749B (0.06 g, 0.5 mmol), toluene (1 mL), triethylamine (0.25 g, 0.35 mL, 2.5 mmol), $MgSO_4$ (0.15 g, 1.3 mmol), and compound 20A. The tube was sealed with a teflon cap and heated overnight at 145° C. The reaction was cooled, diluted with methylene chloride and filtered through Celite. The filtrate was concentrated in vacuo and the residue was purified by column chromatography using 10% ether/methylene chloride as the eluent to give 14 mg (95%) of compound 749C as a light yellow crystalline solid. HPLC: 94.6% at 2.6 minutes (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm. MS (ES): m/z 303.05 $[M+H]^+$.

EXAMPLE 750

(3aα,4β,7β,7aα)-5-[Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-thiophenecarbonitrile (750B)



A. 5-Amino-2-cyanothiophene (750A)



To a 100 mL 3-necked flask was added compound 749Aii (1.63 g, 10.1 mmol) dissolved in ethyl acetate (20 mL) followed by a 10% solution of acetic acid (20 mL). The biphasic mixture was heated to 65° C. and then iron powder (2.95 g, 52.9 mmol) was added portion-wise over 5 minutes. After stirring for 1.5 h at 65° C., the reaction was filtered through a bed of Celite and the pad was washed with ethyl acetate. The organic layer was separated, washed with water (3×20 mL), brine and dried over $MgSO_4$. Filtration and concentration in vacuo gave a dark amber residue which was purified by column chromatography using 10% diethyl ether/methylene chloride as the eluent to give 87 mg (66%) of compound 750A as a brown solid. HPLC: 94.2% at 1.05 minutes (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm).

B. (3aα,4β,7β,7aα)-5-[Octahydro-4,7-dimethyl-1,3-dioxo-4,7-epoxy-2H-isoindol-2-yl]-2-thiophenecarbonitrile (750B)

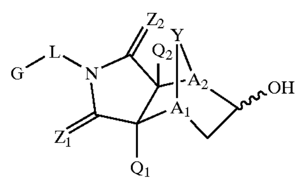
To a Pyrex tube was added compound 750A (0.06 g, 0.5 mmol), toluene (1 mL), triethylamine (0.25 g, 0.35 mL, 2.5

mmol), $MgSO_4$ (0.15 g, 1.3 mmol), and compound 20B. The tube was scaled with a teflon cap and heated overnight at 145° C. The reaction was cooled, diluted with methylene chloride and filtered through Celite. The filtrate was concentrated in vacuo and the residue was purified by column chromatography using 10% ether/methylene chloride as the eluent to yield 143 mg (96%) of compound 750B as a light yellow crystalline solid. HPLC: 92.7% at 2.93 minutes (retention time) (YMC S5 ODS column 4.6×50 mm eluting with 10–90% aqueous methanol over 4 minutes containing 0.2% phosphoric acid, 4 mL/min, monitoring at 220 nm. MS (ES): m/z 303.21 $[M+H]^+$.

We claim:

1. A method for preparation of a compound of the following formula XVI, or salt thereof:

XVI



where

G is an aryl or heterocyclo group, where said group is mono- or polycyclic, and which is optionally substituted at one or more positions;

Z_1 is O or S;

Z_2 is O or S;

A_1 is CR^7;

A_2 is CR^7;

Y is J—J'—J" where J is $(CR^7R^{7'})$n and n=0–3, J' is O, S, S═O, SO_2, NH, NR^7, OP═OOR^2, OC═O, NR^1C═O, OP═$ONHR^2$, OSO_2, NHNH, $NHNR^8$, NR^6NH, or N═N, and J" is $(CR^7R^{7'})$n and n=0–3;

Q_1 is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocycloalkyl or substituted heterocycloalkyl, arylalkyl or substituted arylalkyl, alkynyl or substituted alkynyl, aryl or substituted aryl, heterocyclo or substituted heterocyclo, halo, CN, R^1OC═O, R^4C═O, R^5R^6NC═O, $HOCR^7R^{7'}$, nitro, R^1OCH_2, R^1O, NH_2, C═OSR^1, SO_2R^1 or NR^4R^5;

Q_2 is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocycloalkyl or substituted heterocycloalkyl, arylalkyl or substituted arylalkyl, alkynyl or substituted alkynyl, aryl or substituted aryl, heterocyclo or substituted heterocyclo, halo, CN, R^1OC═O, R^4C═O, R^5R^6NC═O, $HOCR^7R^{7'}$, nitro, R^1OCH_2, R^1O, NH_2, C═OSR^1, SO_2R^1 or NR^4R^5;

L is a bond, $(CR^7R^{7'})$n, NH, NR^5 or $NR^5(CR^7R^{7'})$n, where n=0–3;

R^1 and $R^{1'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkyalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl;

R^2 is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl;

R^4 is H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, R^1C═O, R^1NHC═O, SO_2OR^1, or $SO_2NR^1R^{1'}$;

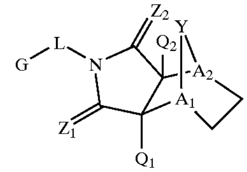
R^5 is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, R^1C═O, R^1NHC═O, SO_2R^1, SO_2OR^1, or $SO_2NR^1R^{1'}$;

R^6 is alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, CN, OH, OR^1, R^1C═O, R^1NHC═O, SO_2R^1, SO_2OR^1, or $SO_2NR^1R^{1'}$; and

R^7 and $R^{7'}$ are each independently H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, cycloalkenyl or substituted cycloalkenyl, heterocyclo or substituted heterocyclo, cycloalkylalkyl or substituted cycloalkylalkyl, cycloalkenylalkyl or substituted cycloalkenylalkyl, heterocycloalkyl or substituted heterocycloalkyl, aryl or substituted aryl, arylalkyl or substituted arylalkyl, halo, CN, OR^1, nitro, hydroxylamine, hydroxylamide, amino, NHR^4, NR^2R^5, NOR^1, thiol, alkylthio or substituted alkylthio, R^1C═O, R^1(C═O)O, R^1OC═O, R^1NHC═O, SO_2R^1, SOR^1, $PO_3R^1R^{1'}$, $R^1R^{1'}NC$═O, C═OSR^1, SO_2OR^1, or $SO_2NR^1R^{1'}$;

comprising the steps of contacting a compound of the following formula XV, or salt thereof:

XV



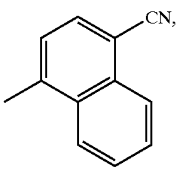
where the symbols are as defined above;

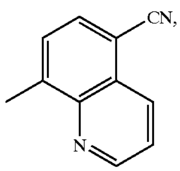
with an enzyme or microorganism capable of catalyzing the hydroxylation of said compound XV to said compound XVI, and effecting said hydroxylation.

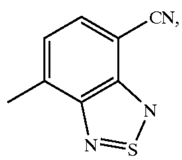
2. The method of claim **1** wherein a microorganism is incubated with the compound of formula XV to effect the hydroxylation.

3. The method of claim **1** wherein the reaction mixture, after hydroxylation, is separated by chiral HPLC.

4. The method of claim **1** wherein R^7 is alkyl or substituted alkyl; L is a bond; and G is selected from the group consisting of;







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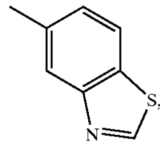

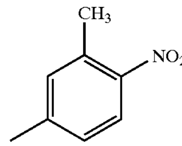

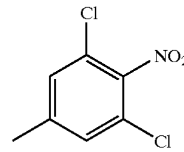

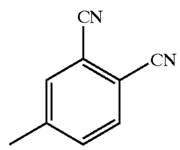

and

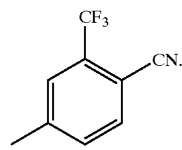

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EXHIBIT H

US006203820B1

(12) **United States Patent**
Vickery

(10) **Patent No.: US 6,203,820 B1**
(45) **Date of Patent: Mar. 20, 2001**

(54) **COMPOSITIONS AND METHODS FOR ENHANCING PROTEIN ANABOLISM AND DETOXIFICATION**

(76) Inventor: **Brice E. Vickery**, 007 W. Ridge Ct., Parachute, CO (US) 81635

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/317,595**

(22) Filed: **May 24, 1999**

Related U.S. Application Data

(63) Continuation of application No. 09/085,845, filed on May 28, 1998, now abandoned.

(51) **Int. Cl.**[7] **A61K 33/26**; A61K 31/415; A61K 31/195

(52) **U.S. Cl.** **424/646**; 514/400; 514/561; 514/562; 514/565

(58) **Field of Search** 424/646; 514/400, 514/561, 562, 565

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Primary Examiner—Raymond Henley, III
(74) *Attorney, Agent, or Firm*—Christie, Parker & Hale, LLP

(57) **ABSTRACT**

A composition for enhancing protein anabolism and detoxification comprises molybdenum and at least two amino acids selected from the group consisting of L-arginine, L-cystine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, and L-valine. Preferably the composition further comprises creatine and/or sulfur. Preferably the amino acids are all free form amino acids. The composition is provided in the form of a powder, which is preferably encapsulated in a gelatin capsule. Methods for enhancing protein anabolism and/or detoxification in a patient comprise administering to the patient an effective amount of a composition as described above. The composition is preferably administered orally in an amount of from about 3 grams/day to about 10.5 grams/day.

43 Claims, 1 Drawing Sheet

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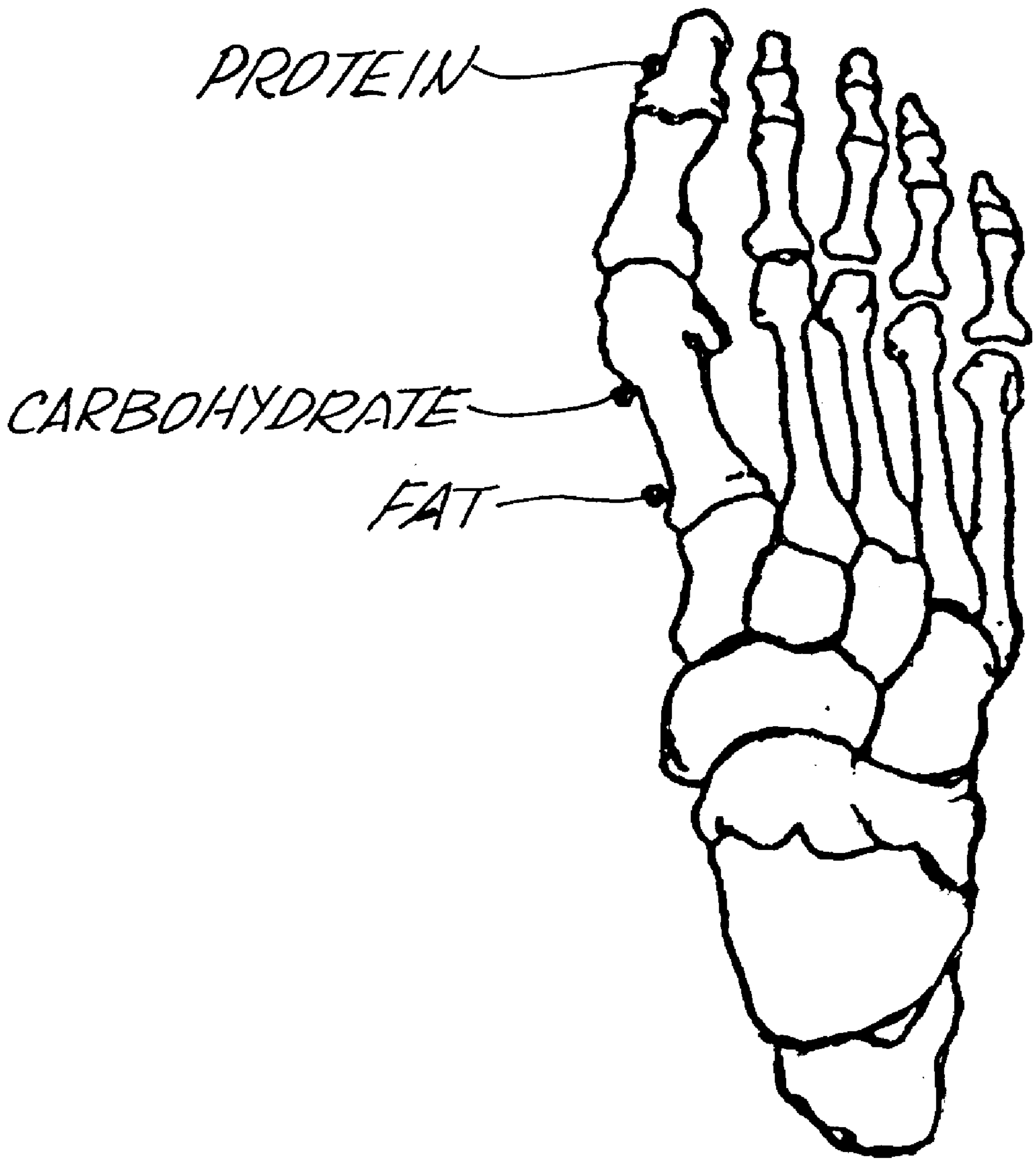
Fig. 1



COMPOSITIONS AND METHODS FOR ENHANCING PROTEIN ANABOLISM AND DETOXIFICATION

This is a continuation of U.S. Application Ser. No. 09/085,845 filed May 28, 1998, now abandoned.

FIELD OF THE INVENTION

The present invention is directed to amino acid compositions useful for enhancing protein anabolism and detoxification.

BACKGROUND OF THE INVENTION

For the last century, the role of vitamins, minerals and essential fatty acids as dietary necessities has been progressively recognized, and supplementation of these nutrients is becoming accepted procedure. Conventional wisdom dictates that dietary intake of between about 30 and 55 grams of protein per day ensures adequate protein for all of the body's functions. In the treatment of protein-deficient patients, it has been found that free form amino acids are superior to maintain sufficient protein levels as compared to protein supplied in complex large molecules. Dietary proteins are not used directly but must be broken down into their constituent individual amino acids in order to be absorbed and utilized by the body. Free form amino acids, in contrast, are individual, single amino acids with no fillers or allergens, and are immediately absorbable by the gut. Upon absorption, the amino acids are carried rapidly in the blood plasma to the cells where they are used or stored. Free amino acids are the building blocks of more complex protein molecules that perform structural or functional roles in tissue. Free form amino acids are presently commercially available.

The amino acid proportions of the blood plasma differ significantly from the profiles of either food proteins or the various body proteins. Pools of amino acids in body tissues are needed for normal anabolic function. This pool is normally replaced by the blood's supply of amino acids. The blood, in turn, draws from pools of free amino acids in other tissues to replace what has been used. Therefore plasma concentrations of amino acids remain relatively constant even the supply through dietary sources is deficient for any reason. Thus, the standard accepted total protein tests of the plasma do not provide an early warning of protein and amino acid deficiencies.

Excess of some amino acids can produce results as detrimental as some deficiencies. Balance in the use of amino acids is of the greatest importance. Thus, a need exists for a superior nutritional formulation that can be used to provide amino acids for enhancing protein anabolism.

Another concern is detoxification, namely, the removal of toxic substances such as alcohol and pesticides from the body. Detoxification occurs in the liver in two phases. During Phase I, the liver cells secrete cytochrome P-450 enzymes, which convert waste products to more soluble forms so that they can be excreted by the colon and kidneys. During Phase II, glutathione and other enzymes in the liver corral free radicals, heavy metals, and some toxic wastes and remove them from the body. Critical to this process is the conversion of sulfites to sulfates. Molybdenum is a component of enzymes that assist in this conversion. Thus, a need also exists for a nutritional formulation that provides a sufficient supply of molybdenum to create the necessary enzymes to assist with Phase II detoxification.

SUMMARY OF THE INVENTION

The present invention is directed to a composition for enhancing protein anabolism. The composition comprises

molybdenum and at least two amino acids selected from the group consisting of L-arginine, L-cystine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, and L-valine. Preferably the composition further comprises creatine monohydrate and/or sulfur, preferably in the form of methylsulfonylmethane.

It is desirable to include all of the above-listed amino acids in the composition. In a particularly preferred embodiment, the composition comprises, based on the total weight of the composition:

- about 6% to about 9% by weight L-arginine,
- about 2% to about 4% by weight L-cystine,
- about 1.5% to about 3.5% by weight L-histidine,
- about 6% to about 9% by weight L-isoleucine,
- about 8% to about 12% by weight L-leucine,
- about 6% to about 8% by weight L-lysine,
- about 2.5% to about 4.5% by weight L-methionine,
- about 5.5% to about 7.5% by weight L-phenylalanine,
- about 4.5% to about 6.5% by weight L-threonine,
- about 4% to about 6% by weight L-tyrosine,
- about 7% to about 10% by weight L-valine,
- about 0.001% to about 0.03% by weight molybdenum,
- about 3% to about 30% by weight creatine monohydrate, and
- about 5% to about 45% by weight methylsulfonylmethane. Preferably the amino acids are all free form amino acids. Alternatively, the amino acids can be provided in the form of powdered egg white or powdered milk, for example, in the event that free form amino acids are not available. When the amino acids are provided in this converted form, the composition preferably further comprises a proteolytic enzyme.

The composition of the invention is provided in the form of a powder, which is preferably encapsulated in a gelatin capsule.

The invention is also directed to a method for enhancing protein anabolism in a patient comprising administering to the patient an effective amount of a composition as described above. The composition is preferably administered orally. Preferably the composition is administered to the patient in an amount of from about 1.5 grams/day to about 15 grams/day, more preferably in an amount of from about 3 grams/day to about 10.5 grams/day.

BRIEF DESCRIPTION OF THE DRAWING

These and other features and advantages of the present invention will be better understood by reference to the following detailed description when considered in conjunction with the accompanying drawing, wherein:

FIG. **1** is an illustration of the Voll Points on the foot, used in the Vickery-Voll Test.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a composition for enhancing protein anabolism. The composition comprises molybdenum and at least two amino acids selected from the group consisting of L-arginine, L-cystine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, and L-valine. Molybdenum activates enzymes in the liver that assist in and enhance the detoxification process. The molyb-

denum can be provided in any suitable form, for example, as sodium molybdate or etrathiolmolybdate. A particularly useful form is Kreb's (available from Douglas Laboratories under the trade name VitaHealthy or from Monarch Nutrition, Utah), which is a complex formed from sodium molybdate, alphaketoglutaric acid, citric acid, fumaric acid, malic acid, succinate and dicalcium phosphate. The molybdenum is present in the composition in an amount of from about 0.001% to about 0.03% by weight, more preferably from about 0.0015% to about 0.01% by weight, based on the total weight of the active ingredients in the composition.

L-Arginine is desirable in the composition for stimulating the immune system and assisting in the utilization of other amino acids. L-Arginine has several other advantages, including blocking the formation of tumors, causing release of growth hormone, forming citrulline and ornithine by hydrolysis, detoxification of ammonia, assisting in liver regeneration, wound healing, growth and sperm formation, and lowering cholesterol. L-Arginine is preferably present in the composition in an amount ranging from about 6% to about 9% by weight, more preferably from about 7% to about 8% by weight, based on the total weight of the active ingredients in the composition.

L-Cystine is convertible to and from cysteine. L-Cystine is believed to minimize the cross-linking of free radicals that age the skin, harden the arteries, deposit aging pigments, and cause arthritis and mutagenic disorders such as cancer. Additionally, L-cystine is the precursor of glutathione, the body's primary antioxidizer and detoxifier that protects against such substances as lead, mercury, radiation, pesticides, and tobacco smoke. L-Cystine is also important in red and white cell formation and facilitating oxygen transport. L-Cystine is preferably present in the composition in an amount ranging from about 2% to 4%, more preferably from about 2.5% to about 3.5%, based on the total weight of the active ingredients in the composition.

L-Histidine is a necessary amino acid for neurotransmitter formation. Additionally, L-histidine regulates blood sugar levels and affects allergic response. L-Histidine is preferably present in the composition in an amount of from about 1.5% to about 3.5% by weight, more preferably from about 2% to about 3% by weight, based on the total weight of the active ingredients in the composition.

L-Isoleucine aids in wound healing and muscle growth. Additionally, L-isoleucine is critical to life in stress, energy and muscle metabolism. L-Isoleucine is preferably present in the composition in an amount of from about 6% to about 9% by weight, more preferably from about 7% to about 8% by weight, based on the total weight of the active ingredients in the composition.

L-Leucine is useful for lowering blood sugar, stimulating protein synthesis in muscle, and wound healing of skin and bone. Additionally, L-leucine stimulates protein synthesis in muscle. L-Leucine is preferably present in the composition in an amount of from about 8% to about 12% by weight, more preferably from about 9% to about 11% by weight, based on the total weight of the active ingredients in the composition.

L-Lysine is a critical enzyme in carbohydrate metabolism. L-Lysine is used in the treatment of herpes viruses and Parkinsons' Psychosis. Additionally, L-lysine builds connective tissue, collagen and bone and assists in calcium transport through the body. L-Lysine is preferably present in the composition in an amount of from about 6% to 8% by weight, more preferably from about 6.5% to about 7.5% by weight, based on the total weight of the active ingredients in the composition.

L-Methionine is a sulfur-containing amino acid that removes heavy metals, quenches free radicals, and lowers cholesterol. L-Methionine also prevents fat accumulation in the liver and, along with cysteine/cystine, helps prevent disorders of the hair, skin, and nails. L-Methionine is preferably present in the composition in an amount of from about 2.5% to 4.5% by weight, more preferably from about 3% to about 4% by weight, based on the total weight of the active ingredients in the composition.

L-Phenylalanine is useful for synthesizing insulin, adrenaline and certain enzymes. Additionally, L-phenylalanine enhances alertness, learning and memory and acts as a pain retardant and appetite suppressant. L-Phenylalanine also helps build collagen and connective tissues. L-Phenylalanine is preferably present in the composition in an amount of from about 5.5% to about 7.5% by weight, more preferably from about 6% to about 7% by weight, based on the total weight of the active ingredients in the composition.

L-Threonine is useful as an immune booster. It degrades into glycine, serine, and glucose. L-Threonine is a wound healer and decreases the harmful effects of aspirin. L-Threonine is preferably present in the composition in an amount of from about 4.5% to about 6.5% by weight, more preferably from about 5% to about 6% by weight, based on the total weight of the active ingredients in the composition.

L-Tryptophan is a precursor to melatonin and serotine. Additionally, L-tryptophan acts as an appetite suppressant, growth hormone stimulant and platelet clotting factor. L-Tryptophan can also be sued to treat insomnia, depression, migraine headaches, and high blood pressure. L-Tryptophan is preferably in the composition in an amount of from about 0.8% to about 3% by weight, more preferably in an amount of from about 1.5% to about 2.5% by weight, based on the total weight of the active ingredients in the composition. If desired, 5-hydroxytryptophan can be used instead of L-tryptophan.

L-Tyrosine is a precursor for dopamine, norepinephrine, thryoxin, catacholestrogens, melanin, and enkephalines. L-Tyrosine can be used as an antidepressant, a growth hormone promoter, an appetite suppressant, or an antioxidant. L-Tyrosine is preferably present in the composition in an amount of from about 4% to about 6% by weight, more preferably from about 4.5% to about 5.5% by weight, based on the total weight of the active ingredients in the composition.

L-Valine aids in would healing, muscle growth and liver diseases. L-Valine is preferably present in the composition in an amount of from about 7% to about 10% by weight, more preferably from about 8% to about 9% by weight, based on the total weight of the active ingredients in the composition.

If available as both a base and a hydrochloride, each amino acid is preferably used in the base form. The amino acids are preferably in the form of free form amino acids. Alternatively, the amino acids can be provided in converted form. The converted amino acids can be supplied, for example, in the form of egg white powder or milk powder. If the amino acids are provided in converted form, preferably the composition further comprises a proteolytic enzyme in an amount of from about 15% to about 25%, based on the total weight of the active ingredients in the composition.

Preferably the composition contains at least three, more preferably at least four, still more preferably at least five, and even more preferably at least six, of the above-listed amino acids. In a particularly preferred embodiment, the compo-

sition contains all of the above-listed amino acids. In another particularly preferred embodiment, the composition contains all of the above-listed amino acids except L-tryptophan.

Preferably the composition further comprises creatine. Creatine is a natural body substance found to be distributed primarily in the skeletal muscle. Creatine assists in the replenishment of adenosine triphosphate, an energy source used in protein synthesis, in the muscles. Creatine has no known side effects. Preferably the creatine is in the form of creatine monohydrate. Preferably the creatine is present in the composition in an amount of from about 3% to about 30% by weight, more preferably from about 12% to about 22% by weight, based on the total weight of the active ingredients in the composition.

Preferably the composition also comprises sulfur in addition to the sulfur provided in any of the amino acids. Sulfur is present in every cell of the body and is necessary for collagen synthesis. The body turns over approximately 850 mg of sulfur a day, resulting in a daily deficit. Food alone cannot always overcome this deficit. The sulfur can be provided in any suitable form, for example, as methylsulfonylmethane. Preferably sulfur is present in the composition in an amount of from about 1.5% to about 15%, more preferably from about 5% to about 10% by weight, based on the total weight of the active ingredients in the composition. If methylsulfonylmethane is used as the source of sulfur, preferably the methylsulfonylmethane is present in the composition in an amount of from about 5% to about 45% by weight, more preferably from about 15% to about 30% by weight, based on the total weight of the active ingredients in the composition.

A particularly preferred composition according to the invention comprises about 6% to about 9% by weight L-arginine, about 2% to about 4% by weight L-cystine, about 1.5% to about 3.5% by weight L-histidine, about 6% to about 9% by weight L-isoleucine, about 8% to about 12% by weight L-leucine, about 6% to about 8% by weight L-lysine, about 2.5% to about 4.5% by weight L-methionine, about 5.5% to about 7.5% by weight L-phenylalanine, about 4.5% to about 6.5% by weight L-threonine, about 4% to about 6% by weight L-tyrosine, about 7% to about 10% by weight L-valine, about 0.001% to about 0.03% by weight molybdenum, about 3% to about 30% by weight creatine, about 5% to about 45% by weight methylsulfonylmethane, and optionally about 0.8% to about 3% by weight L-tryptophan or 5-hydroxytryptophan, based on the total weight of the active ingredients in the composition.

Another composition according to the invention comprises about 40% to about 55% by weight powdered egg white or powdered milk, about 15% to about 25% by weight of a proteolytic enzyme, about 20% to about 45% by weight methylsulfonylmethane, about 5% to about 15% by weight creatine, and from about 0.0015% to about 0.0050% by weight molybdenum, based-on the total weight of the active ingredients in the composition.

As used herein, the term "active ingredients" refers to the following: L-arginine, L-cystine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, L-valine, molybdenum, creatine, creatine monohydrate, sulfur, and methylsulfonylmethane. When the term "active ingredients" is used in connection with a composition comprising powdered egg white or powdered milk, the term also encompasses powdered egg white, powdered milk, and proteolytic enzymes.

The compositions according to the invention can be made by any suitable method known to those skilled in the art. For example, the free form amino acids, as well as the methylsulfonylmethane, creatine monohydrate, and molybdenum, are provided in the form of a powder. Similarly, the powdered egg white, powdered milk and proteolytic enzyme are available in powder form. For relatively small batches (e.g., less than 10 kilos), the desired amount of each component is measured and put into a large plastic bag. The plastic bag is sealed with a large amount of air remaining in the bag. The bag is then shaken and tossed for about ten minutes to thoroughly mix the composition. For larger batches, the components can be mixed in a large stainless steel mixer.

The blended composition can then be provided in any suitable dosage form. For example, if an oral dosage form is desired, the composition can be contained within gelatin capsules, preferably ones that do not deteriorate at room temperature, such as those made from beef or pork gelatin. Other suitable solid dosage forms can be used, including as tablets, capsules, caplets, granules, and bulk powders. Tablets can be compressed, tablet triturates, enteric-coated, sugar-coated, film-coated, multiply compressed or multiply layered containing suitable binder, lubricants, diluents, disintegrating agents, coloring agents, flavoring agents, and preservatives. Liquid dosage forms include aqueous and nonaqueous solutions, emulsions, suspensions and solutions and/or suspensions reconstituted from non-effervescent granules, containing suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, coloring agents, and flavoring agents. Specific examples of pharmaceutically acceptable carriers and excipients that may be used to formulate oral dosage forms are described in the Handbook of Pharmaceutical Excipients, American Pharmaceutical Associated (1986), the disclosure of which is incorporated herein by reference. Techniques and compositions for making tablets, capsules and pills are described in Remington's Pharmaceutical Sciences (Arthur Oxol, ed. 1980), the disclosure of which is incorporated herein by reference.

The compositions can be administered to a patient by any suitable method, and are preferably administered orally or rectally. As used herein, the term "patient" refers to any mammal, including humans, dogs and cats. A preferred dosage for adult humans is from about 1.5 grams/day to about 15 grams/day, more preferably from about 3 grams/day to about 10.5 grams/day, of the composition.

EXAMPLES

In the examples, the following tests were used:

Vickery-Voll Test:

The Vickery-Voll test is a simple test used to determine amino acid deficiencies. The three Voll Points are localized on the right foot, as shown in FIG. **1**. A finger is placed on each point, one point at a time, and the right tensor fascia muscle is tested for weakening. Weakening of the muscle yields a positive result, indicating amino acid deficiencies.

Hypoglycemic Test (HOG)

The HOG test is also used to determine amino acid deficiencies. The patient is placed on his back, with his left leg extended and raised to 45 degrees. The leg muscle is tested for strength. A positive result, i.e., weakness, indicates inferred amino acid deficiencies and potential or active hypoglycemic state.

Gland Scan Test

The Gland Scan test is another amino acid deficiency test. The patient places his hands on Neuro Lymphatic points and alarm points. The patient's reflexes are read. Weakening of the muscle corroborates amino acid deficiencies and electrical polarity change in the glands tested.

BEV Test

The BEV test is used to determine the presence of spinal disk lesions. Pressure is exerted on different areas of the patient's spine (cervical, lumbar and dorsal regions), and the patient bends his spine in each region in four different quadrants. The mid-deltoid muscle is tested for strength, with weakening evidencing the presence of spinal disk lesions. The BEV test is described in more detail in Vickery, The Collected Papers of the International College of Applied Kinesiology, Summer 1990–91, Vol. I, pages 224–234, the disclosure of which is incorporated herein by reference.

Confirmatory Challenge Test (CCT)

The Confirmatory Challenge Test is also used to determine the presences of disk lesions. A description of the Confirmatory Challenge Test is provided in Vickery, Collected Papers of the Members of the International College of Applied Kinesiology-U.S.A., Summer 1989–90, Vol. I, pages 259–266, the disclosure of which is incorporated herein by reference.

Raglan Test

The Raglan Test is used to measure adrenal insufficiency. The patient's blood pressure is taken sitting, standing, and laying down. A drop in blood pressure when the patient goes from laying to sitting or from sitting to standing indicates adrenal insufficiency.

Example 1

A composition was prepared by combining the following:

L-arginine base	57 mgs
L-cystine	20 mgs
L-histidine base	19 mgs
L-isoleucine	58 mgs
L-leucine	75 mgs
L-lysine HCl	53 mgs
L-methionine	27 mgs
L-phenylalanine	49 mgs
L-threonine	42 mgs
L-tyrosine	37 mgs
L-valine	64 mgs
molybdenum*	15 mcgs
creatine monohydrate	125 mgs
methylsulfonylmethane	125 mgs

*The molybdenum was provided by Douglas Laboratories as a molybdenum composition comprising primarily dicalcium phosphate with a molybdenum complex containing sodium molybdate combined with alphaketoglutaric acid, citric acid, fumaric acid, malic acid, and succinate, to achieve a total molybdenum concentration of 1% by weight.

The components, all in powdered form, were combined in a plastic bag, which was filled with air and sealed closed. The bag was tossed and shaken for approximately 10 minutes. The mixed powder was then put into gel capsules using an encapsulation machine.

Example 2

The patient had a lumbar facet risotomy in 1990 and left knee reconstruction in 1976 and was diagnosed with Crohn's disease. An MRI revealed disk herniation between L4–L5. Additionally, the patient had burning pain down the right leg (front) and severe back pain. Surgery was scheduled two days later.

Examination revealed that the L4–L5 lesion was inactive. CCT tests revealed that T12-L1, L1–L2, L2–L3 disks were actively causing the condition. The subject was also found to have a protein deficiency, along with other nutritional deficiencies, and degenerating disks in the thoracic and cervical spine (a common finding) using the BEV tests. The subject was placed on the composition described in Example 1 six times per day, along with other nutrients, namely NESS Formula 11 (an enzyme C Complex, NESS, Riverside, Mo.), Multi Plus (multi-vitaminerals by Biotics Research Corp., Houston, Tex.), and Flax/Borage Oil (Pure Encapsulations, Sudbury, Mass.). No spinal correction was done. The patient experienced so much pain relief and healing in the two days that the operation was canceled. The patient had two chiropractic adjustments within a week. No surgery was performed, and follow-up examination one month later revealed no diskal degeneration anywhere in the spine, no sign of the Crohn's disease, and that straight posture was being maintained.

Example 3

A patient was troubled by fungus infection under both large toenails that no spray, ointment, or cream seemed to mitigate, despite a daily consumption of three grams of an enhanced amino acid formulation (containing all of the amino acids in the formulation of Example 1, but no molybdenum, sulfur or creatine monohydrate) and a full nutritional supplement spectrum of professional quality. Five months after starting on the formula described in Example 1 six to eight times per day, both toenails were pink and normal. The toenails were also softer and grew faster.

Example 4

A fifty-year-old patient was seen in 1998 for severe pains in the left shoulder, neck, and right ankle. He had previously had surgery that involved removal of bone on the left scapula with muscular reattachment. He had also had surgery on his left temporomandibular joint (TMJ) in 1982, and cervical disk surgery in 1991. Examination revealed that every BEV Test was positive throughout the spine and that the spine was in a state of severe degeneration. He also had persistent muscle injury in his left scapula, neck and right ankle. Bacterial infection was found at the neck, left scapula and the right ankle. He tested positive for Epstein Barr Virus, was vitamin C deficient (miniscurvy), and was antioxidant deficient. He also tested positive for hypoglycemia (HOG) and hyperglycemia (HYG). He was placed on a composition as described in Example 1 six times per day, Bio Multi Plus (multi-vitaminerals by Biotics Research Corp., Houston, Tex.) three times per day, Biotics Bioprotect (antioxidant formula, Biotics Research Corp.) four times per day, and NESS Formula 11 (an enzyme C Complex, NESS, Riverside, Miss.) six times per day. Within one week, the Vickery-Voll Test was negative, most of the spinal disks were healing, the infections were healing and 75% of the pains had disappeared.

Example 5

A 50-year-old female patient complained about universal allergic reaction, being subject to immediate whole body swelling, high blood pressure, being overweight, various aches and pains, headaches, and skin eruptions. She was

house-bound for ten years, and her home was kept cold to avoid furnace fumes. She engaged in no physical activity and had a limited diet. She previously was athletic, having bicycled across the country, and considered becoming a professional golfer. Suddenly she became ill in 1986 and deteriorated thereafter. She saw many doctors and had all silver amalgam fillings removed.

She was examined, and BEV tests were all positive in every area of the spine, indicating severe tissue degeneration. The Vickery-Voll, HOG, Gland Scan, and Raglan tests were all positive. Epstein Barr Virus, yeast infection throughout the body, and vitamin C, fatty acid, calcium, NaCl, and B vitamin deficiencies were found. Parasites were suspected. Sensitivity to silver amalgam and formaldehyde was positive. The patient was placed on a formulation similar to that described in Example 1, but without the molybdenum, sulfur, or creatine monohydrate at an increasing rate (2/day-4/day-6/day) as tolerated, and all other deficiencies were addressed. Slow and stair-like progress took place with many exacerbations and remissions. In 1997, the patient began on a formulation as described in Example 1, beginning with one 750 mg capsule per day, gradually increasing to four capsules per day. The patient is now walking two miles a day and able to enter stores, hotels, and shopping malls. The patient now practices golf and will soon be playing again. This case demonstrates the increased detoxification ability of the formulation of Example 1 through increased sulfate metabolism in the liver as well as its tissue rebuilding aspect.

The above descriptions of exemplary embodiments of compositions and methods for enhancing protein anabolism are for illustrative purposes. Because of variations which will be apparent to those skilled in the art, the present invention is not intended to be limited to the particular embodiments described above. The scope of the invention is defined in the following claims. Further, it should be understood that the composition of the invention can function in accordance with the practice of the invention in the absence of any elements or materials not specifically described herein as being part of the composition.

I claim:

1. A composition for enhancing protein anabolism or detoxification, comprising molybdenum, at least one non-amino acid source of sulfur, and at least two amino acids selected from the group consisting of L-arginine, L-cystine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, and L-valine, wherein the composition is in oral or rectal dosage form.

2. A composition according to claim **1**, wherein the molybdenum is present in the composition in an amount of from about 0.001% to about 0.03% by weight, based on the total weight of the active ingredients in the composition.

3. A composition according to claim **1**, wherein the molybdenum is present in the composition in an amount of from about 0.0015% to about 0.01% by weight, based on the total weight of the active ingredients in the composition.

4. A composition according to claim **1**, further comprising creatine.

5. A composition according to claim **4**, wherein the creatine is present in the composition in an amount of from about 3% to about 30% by weight, based on the total weight of the active ingredients in the composition.

6. A composition according to claim **4**, wherein the creatine is present in the composition in an amount of from about 12% to about 22% by weight, based on the total weight of the active ingredients in the composition.

7. A composition according to claim **1**, wherein the at least one non-amino acid source of sulfur is present in the composition in an amount of from about 1.5% to about 15%, based on the total weight of the active ingredients in the composition.

8. A composition according to claim **1**, wherein the at least one non-amino acid source of sulfur is present in the composition in an amount of from about 5% to about 10%, based on the total weight of the active ingredients in the composition.

9. A composition according to claim **1**, comprising methylsulfonylmethane.

10. A composition according to claim **9**, wherein the methylsulfonylmethane is present in the composition in an amount of from about 5% to about 45% by weight, based on the total weight of the active ingredients in the composition.

11. A composition according to claim **9**, wherein the methylsulfonylmethane is present in the composition in an amount of from about 15% to about 30% by weight, based on the total weight of the active ingredients in the composition.

12. A composition according to claim **4**, further comprising methylsulfonylmethane.

13. A composition according to claim **1**, comprising at least three amino acids selected from the group consisting of L-arginine, L-cystine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, and L-valine.

14. A composition according to claim **1**, comprising at least four amino acids selected from the group consisting of L-arginine, L-cystine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, and L-valine.

15. A composition according to claim **14**, further comprising creatine.

16. A composition according to claim **1**, comprising methylsulfonylmethane, L-arginine, L-cystine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tyrosine, and L-valine, and further comprising creatine monohydrate.

17. A composition according to claim **16**, further comprising L-tryptophan or 5-hydroxytryptophan.

18. A composition according to claim **16**, comprising, based on the total weight of the active ingredients in the composition:

about 6% to about 9% by weight L-arginine,
about 2% to about 4% by weight L-cystine,
about 1.5% to about 3.5% by weight L-histidine,
about 6% to about 9% by weight L-isoleucine,
about 8% to about 12% by weight L-leucine,
about 6% to about 8% by weight L-lysine,
about 2.5% to about 4.5% by weight L-methionine,
about 5.5% to about 7.5% by weight L-phenylalanine,
about 4.5% to about 6.5% by weight L-threonine,
about 4% to about 6% by weight L-tyrosine,
about 7% to about 10% by weight L-valine,
about 0.001% to about 0.03% by weight molybdenum,
about 3% to about 30% by weight creatine monohydrate, and
about 5% to about 45% by weight methylsulfonylmethane.

19. A composition according to claim **18**, further comprising L-tryptophan or 5-hydroxytryptophan in an amount of from about 0.8% to about 3% by weight, based on the total weight of the active ingredients in the composition.

20. A composition according to claim 1, comprising, based on the total weight of the active ingredients in the composition:

about 7.3% by weight L-arginine,
about 2.7% by weight L-cystine,
about 2.5% by weight L-histidine,
about 7.5% by weight L-isoleucine,
about 9.7% by weight L-leucine,
about 6.9% by weight L-lysine,
about 3.5% by weight L-methionine,
about 6.4% by weight L-phenylalanine,
about 5.5% by weight L-threonine,
about 4.8% by weight L-tyrosine,
about 8.3% by weight L-valine,
about 1.9% tryptophan or 5-hydroxytryptophan,
about 0.002% by weight molybdenum,
about 16.6% by weight creatine monohydrate, and
about 16.6% by weight methylsulfonylmethane.

21. A composition according to claim 1, comprising, based on the total weight of the active ingredients in the composition:

about 7.6% by weight L-arginine,
about 2.7% by weight L-cystine,
about 2.5% by weight L-histidine,
about 7.7% by weight L-isoleucine,
about 10% by weight L-leucine,
about 7.1% by weight L-lysine,
about 3.6% by weight L-methionine,
about 6.5% by weight L-phenylalanine,
about 5.6% by weight L-threonine,
about 4.9% by weight L-tyrosine,
about 8.5% by weight L-valine,
about 0.002% by weight molybdenum,
about 16.6% by weight creatine monohydrate, and
about 16.6% by weight methylsulfonylmethane.

22. A composition according to claim 1, wherein the amino acids are free form amino acids.

23. A composition according to claim 18, wherein the amino acids are free form amino acids.

24. A composition according to claim 1, wherein the at least two amino acids are contained in powdered egg white or powdered milk.

25. A composition according to claim 24, further comprising a proteolytic enzyme.

26. A composition according to claim 25, further comprising creatine monohydrate.

27. A composition according to claim 1 in the form of a powder.

28. A composition according to claim 27, wherein the powder is encapsulated in a gelatin capsule.

29. A method for enhancing protein anabolism in a patient, comprising administering to the patient an effective amount of a composition according to claim 1.

30. A method according to claim 29, wherein the composition is administered to the patient in an amount of from about 1.5 grams/day to about 15 grams/day.

31. A method according to claim 29, wherein the composition is administered to the patient in an amount of from about 3 grams/day to about 10.5 grams/day.

32. A method according to claim 29, wherein the following total amounts of active ingredients are administered each day to the patient:

L-arginine: about 0.18 to about 0.95 grams/day,
L-cystine: about 0.06 to about 0.42 grams/day,
L-histidine: about 0.05 to about 0.37 grams/day,
L-isoleucine: about 0.18 to about 0.95 grams/day,
L-leucine: about 0.24 to about 1.26 grams/day,
L-lysine: about 0.18 to about 0.84 grams/day,
L-methionine: about 0.07 to about 0.47 grams/day,
L-phenylalanine: about 0.15 to about 0.79 grams/day,
L-threonine: about 0.13 to about 0.68 grams/day,
L-tyrosine: about 0.12 to about 0.63 grams/day,
L-valine: about 0.21 to about 1.05 grams/day,
molybdenum: about 0.03 to about 3.15 mg/day,
creatine: about 0.09 to about 3.15 grams/day, and
sulfur: about 0.15 to about 4.73 grams/day.

33. A method for enhancing detoxification in a patient, comprising administering to the patient an effective amount of a composition according to claim 1.

34. A method according to claim 33, wherein the composition is administered to the patient in an amount of from about 1.5 grams/day to about 15 grams/day.

35. A method according to claim 3, wherein the composition is administered to the patient in an amount of from about 3 grams/day to about 10.5 grams/day.

36. A method according to claim 33, wherein the following total amounts of active ingredients are administered each day to the patient:

L-arginine: about 0.18 to about 0.95 grams/day,
L-cystine: about 0.06 to about 0.42 grams/day,
L-histidine: about 0.05 to about 0.37 grams/day,
L-isoleucine: about 0.18 to about 0.95 grams/day,
L-leucine: about 0.24 to about 1.26 grams/day,
L-lysine: about 0.18 to about 0.84 grams/day,
L-methionine: about 0.07 to about 0.47 grams/day,
L-phenylalanine: about 0.15 to about 0.79 grams/day,
L-threonine: about 0.13 to about 0.68 grams/day,
L-tyrosine: about 0.12 to about 0.63 grams/day,
L-valine: about 0.21 to about 1.05 grams/day,
molybdenum: about 0.03 to about 3.15 mg/day,
creatine: about 0.09 to about 3.15 grams/day, and
sulfur: about 0.15 to about 4.73 grams/day.

37. A composition according to claim 1, wherein the composition is in oral dosage form.

38. A composition for enhancing protein anabolism or detoxification, comprising molybdenum, creatine and at least two amino acids selected from the group consisting of L-arginine, L-cystine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, and L-valine.

39. A composition according to claim 38, wherein the molybdenum is present in the composition in an amount of from about 0.001% to about 0.03% by weight, based on the total weight of the active ingredients in the composition.

40. A composition according to claim 38, wherein the creatine is present in the composition in an amount of from about 3% to about 30% by weight, based on the total weight of the active ingredients in the composition.

41. A method for enhancing protein anabolism or detoxification in a patient, comprising administering to the patient an effective amount of a composition according to claim 38.

42. A method according to claim 41, wherein the composition is administered to the patient in an amount of from about 1.5 grams/day to about 15 grams/day.

43. A method according to claim 41, wherein the composition is administered to the patient in an amount of from about 3 grams/day to about 10.5 grams/day.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,203,820 B1
DATED : March 20, 2001
INVENTOR(S) : Brice E. Vickery

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page,
Item [56], **References Cited**, OTHER PUBLICATIONS, "Erdmann, R. et al.," replace "Change the Way You Fell" with -- Change the Way You Feel --, "B.M. Cohen et al.," replace "alpha 1-nad beta adrenoceptors" with -- alpha 1-and beta-adrenoceptors --.

Column 12,
Line 20, replace "claim **3**" with -- claim **33** --.

Signed and Sealed this

Second Day of July, 2002

Attest:

JAMES E. ROGAN

Attesting Officer

Director of the United States Patent and Trademark Office

EXHIBIT I

US 20030119888A1

(19) United States
(12) Patent Application Publication (10) Pub. No.: US 2003/0119888 A1
ALLEN (43) Pub. Date: Jun. 26, 2003

(54) **COMPOSITION COMPRISING L-ARGININE AS A MUSCLE GROWTH STIMULANT AND USE THEREOF**

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(*) Notice: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

(21) Appl. No.: **08/938,801**

(22) Filed: **Sep. 26, 1997**

Related U.S. Application Data

(63) Continuation of application No. 08/784,132, filed on Jan. 15, 1997, now abandoned, which is a continuation of application No. 08/562,395, filed on Nov. 24, 1995, now abandoned, which is a continuation of application No. 08/215,667, filed on Mar. 22, 1994, now abandoned, which is a continuation of application No. 07/793,837, filed on Nov. 20, 1991, now abandoned.

Publication Classification

(51) **Int. Cl.**[7] **A61K 31/42**; A01N 43/80
(52) **U.S. Cl.** **514/380**

(57) **ABSTRACT**

A composition for stimulating muscle growth, comprising an effective amount of L-arginine or a salt thereof, a pH control agent for controlling the pH at less than 7 and a pharmaceutically acceptable carrier. A method of using the composition to achieve muscle growth or an immune response is also described.

COMPOSITION COMPRISING L-ARGININE AS A MUSCLE GROWTH STIMULANT AND USE THEREOF

BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention

[0002] The present invention relates to a novel composition useful as a replacement for steriods as a muscle growth stimulant and for stimulating an immure response, and to the use thereof.

[0003] 2. Description of the Prior Art

[0004] The problems of maintaining muscle mass while minimizing the accumulation of fat has long been an issue of concern to athletes. While exercise is one of the main mechanisms for achieving that goal, food and/or vitamin supplements, as well as pituitary growth hormone, are necessary for muscle growth. Such ergogenic aids, that is supplements which stimulate muscle growth, include the three amino acids, leucine, isoleucine and valine. Human Growth Hormone (HGH) has also been considered. Zale, N., "Amino Acids—Growth Hormone Stimulants Fact or Fiction?", *Iron Man Magazine*, January 1985 and New England Journal of Medicine (1990). Growth hormone produces an anabolic effect which includes the process of converting amino acids into protein. The cost of using human growth hormone is very high. Thus, economic reality generally detracts from its use.

[0005] Likewise, the use of steroids to improve performance and increase muscle mass is fraught with disadvantages. Anabolic steroids have undesirable side effects which are linked to a variety of serious health problems including cardiovascular disease and liver cancer.

[0006] Chemical compounds have also been found to be important not for their direct influence on muscle growth, but rather as complements to or synergists for other compounds which stimulate muscle growth. Boron is one such compound. Boron is an essential mineral which is believed to play an important role in the retention of calcium. Likewise, chromium has been found to be a beneficial supplement for athletes. It has been speculated that chromium losses are twice as high on a workout day versus a non-workout day.

[0007] In addition, trans ferulic acid, which is the metabolically active form of gamma oryzanol, is believed to increase the production of key brain chemicals thereby causing increased secretion of growth hormone.

[0008] In spite of the extensive studies and research to date, there remains a long-felt need in the art for a safe and effective method of stimulating muscle growth and taking the place of steroids, especially for athletes.

SUMMARY OF THE INVENTION

[0009] Surprisingly, the present inventor has now found that the laevorotatory form of arginine (L-arginine) is useful as a muscle stimulant in a mammalian organism, especially a human, when preferably present in a formulation which contains the proper adjuvants and/or synergists. More particularly, it has been found that therapeutic doages of L-arginine, typically up to 15 g per day, can be rendered more palatable to a patient (L-arginine has an extremely unpleasant taste) and are better physiologically tolerated by a patient (reduced incidence of diarrhea, headache, flatulence, and depletion of vitamins and electrolytes) by careful control of the pH of the formulation so as to be less than 7, i.e. an acid pH. As a result, long term therapy with L-arginine with reduced side-effects is obtained, in particular reduced depletion of acetyl choline from the brain and consequential reduced incidence of memory loss.

[0010] In one aspect, the present invention provides a composition comprising L-arginine in a form which is palatable to a patient, physiologically tolerated and suitable for enhancing muscle growth, body fat reduction and stimulation of growth hormone in a mammalian organism, in association with a pH control agent for controling the pH of the composition at less than 7, preferably less than 6.0, for example about 3.5 to 5.5, especially 4.5 to 4.7, and an pharmaceutically acceptable carrier.

[0011] More particularly, the present invention provides a composition or complex comprising L-arginine, L-leucine, L-isoleucine, L-valine, boron (or sodium borate), vitamin B5 (calcium pantothenate), chromium, trans ferulic acid, gamma oryzanol, choline, fructose, lemon, lime and citric acid, in which the ratio of components is such that the pH of the composition is less than 7.0, preferably less than 6.0, more preferably 4.5 to 4.7. The composition suitably allows enhanced muscle growth with minimum side effects, especially over extended therapy periods, for example 4 to 8 weeks.

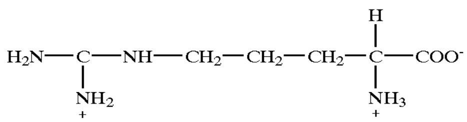
[0012] In another aspect, the invention provides a method for stimulating muscle growth in a mammalian organism comprising the step of administering to a host a muscle growth stimulant or enhancing effective amount of an L-arginine formulation comprising L-arginine or a salt thereof in association with a pH control agent for controling the pH of the formulation at less than 7 and an adjuvant suitable for stimulating muscle growth.

[0013] In yet another aspect, there is provided a method of stumulating an immune response in a patient in need of such treatment, comprising the step of administering to that patient an effective amount of an L-arginine formulation in association with a suitable adjuvant and/or diluent. Preferably, the L-arginine is administered intravenously as an aqueous solution in an amount of 1-10 g per day. More preferably, the L-arginine is co-administered with an immune system stimulator which is preferably vitamin C, in an amount of 1-10 g per day.

[0014] Other objects and advantages of the present invention will be apparent from the following description.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0015] Arginine, 2-amino-5-guanidinovaleric acid, is a basic amino acid with a positively charged guanidinium group. The IUPAC abbreviation is Arg. Arginine can be depicted as follows.



[0016] Arginine is considered to be a semi-essential amino acid. It can be synthesized in animal tissue at a rate sufficient for maintenance in the adult but not rapidly enough to support growth in the young animal. It is thus an essential amino acid for growth but not for maintenance. It is difficult to obtain therapeutic amounts in chicken and turkey, thus the food supplement of arginine.

[0017] In the mammalian body, arginine takes part in the formation of urea yielding ornithine. Arginine may be synthesized in the mammalian body from alpha-ketoglutaric acid, glutamic acid or proline.

[0018] In addition to L-arginine free base, any salt of L-arginine is suitable in the practice of the present invention. Such salts include L-arginine hydrochloride and the like. L-arginine hydrochloride is the preferred salt in the practice of the present invention.

[0019] Additional suitable anions for such a salt of L-arginine include bromide, fluoride, iodide, borate, hypobromite, hypochlorite, nitrite, nitrate, hyponitrite, sulfate, disulfate, sulfite, sulfonate, phosphate, diphosphate, phosphite, phosphonate, diphosphonate, perchlorate, perchlorite, oxalate, malonate, succinate, lactate, carbonate, bicarbonate, acetate, benzoate, citrate, tosylate, permanganate, manganate, propanolate, propanoate, ethandioate, butanoate, propoxide, chromate, dichromate, selenate, orthosilicate, metasilicate, pertechnetate, technetate, dimethanolate, dimethoxide, thiocyanate, cyanate, isocyanate, 1,4-cyclohexanedithiolate, oxidobutanoate, 3-sulfidocyclobutane-1-sulfonate, 2-(2-carboxylatoethyl)-cyclohexanecarboxylate, 2-amino-4-(methythio)-butanoate and the like. The suitable cation for most salts is hydrogen. However, other cations such as sodium, potassium and the like would be acceptable in the preparation of such a salt.

[0020] L-arginine free base is, however, preferred since it is less hydroscopic and therefore more stable. Free form amino acids are assimilated in an entirely different manner than amino acids derived from foods containing protein. The free form allows for almost immediate elevation of the level of amino acids in the blood plasma. Further, it would be essential to take the L-arginine on an empty stomach so as to minimize any competition between L-arginine and protein in the stomach.

[0021] The precise amount of L-arginine suitable for use in the practice of the present invention will vary depending on the adjuvants or synergists present, the size and kind of the mammal and the specific form, i.e., salt or base, selected.

[0022] The typical muscle growth stimulating or enhancing effective amount of L-arginine or acceptable salt thereof would be in the range of about 1.0 grams to 60 grams per day, preferably about 3.0 grams to 30 grams per day.

[0023] The L-arginine or salt thereof may be administered to a mammalian organism by any route of administration. Suitable routes would, of course, include oral, parenteral, topical, and the like. The oral dosage form is preferred in a non-tablet, non-capsule free form powder.

[0024] The present inventor has found that while L-arginine or salt thereof is useful in stimulating muscle growth, the L-arginine when taken alone on an empty stomach has some disadvantages. First, the L-arginine has an extremely unpleasant taste which makes the oral administration of large dosages difficult and sometimes impossible. Other disadvantages arising from the ingestion of L-arginine include headache, diarrhea, flatulence, depletion of electrolytes, as well as depletion of vitamins, minerals and the like. The side effects from the administration of L-arginine by itself vary significantly from individual to individual and run the gamit of mild to severe.

[0025] In order to overcome these disadvantages, the present inventor has discovered that it is possible to administer hugh dosages of L-arginine if the formulation has an acid pH, i.e. less than 7.0. The preferred pH is less than 6.0, for example 4.3 to 4.8, suitably 4.5 to 4.7. By controlling the pH in this way, it has been found that the L-arginine is more readily accepted by the gastrointestinal tract, that there is minimum depletion of normal body chemicals and is pleasant to taste. Moreover, the stability of the composition is improved as a result of ensuring that the composition has an acid pH.

[0026] The pH is preferably controlled by the presence in the L-arginine composition of a pharmaceutically acceptable acid in an amount which results in a pH of 7 or less when the composition is administered orally. A preferred acid is citric acid, but the invention is not limited to this acid and other suitable pharmaceutically acceptable acids may be employed.

[0027] The pH control agent is present in an amount so as to achieve the desired pH of less then 7. Preferably, the weight ratio of pH control agent to L-arginine in the composition is 0.001-1 pH control agent: 1 L-arginine, for example 0.5-0.9 pH control agent: 1 L-arginine. Examples of weight ranges are 0.001 mg -100 mg per 1.0 to 60 g of L-argenine, typically 2.0 to 5.0 g per 6 g of L-argenine.

[0028] A preferred formulation is set forth below:

	Preferred Dosage Range	Daily Preferred Dosage
L-Arginine (free base)	1.0–60.0 g	30.0 g
L-Leucine	25–200 mg	100.0 mg
L-Isoleucine	25–200 mg	50.0 mg
L-Valine	25–200 mg	50.0 mg
Boron (Sodium Borate)	1.0–30 mg	5.0 mg
Vitamin B5 (Calcium Pantothenate)	10–100 mg	50.0 mg
Chromium	10–50 mg	25.0 mg
Trans Ferulic Acid	5.0–100 mg	10.0 mg
Gamma Oryzanol	5.0–100 mg	15.0 mg
Choline	10.0–700 mg	50.0 mg
Fructose	2.0–10.0 g	6.0 g
Lemon (Natural)	0.1–1.0 mg	0.5 mg
Lime (Natural)	0.1–5.0 mg	1.0 mg
Citric Acid	1.0–10.0 mg	5.0 g

[0029] It is understood, however, that certain substitutions, deletions and additions of other ingredients would still

provide the benefits of the present invention. Thus, all equivalents of the formula set forth above are intended to be encompassed by the scope of the claims.

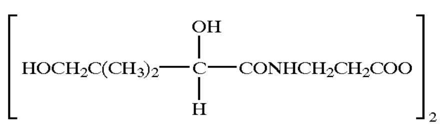
[0030] While the L-arginine described above has been described as the free base, any suitable salt thereof is useful in the practice of the present invention.

[0031] The precise components of the formula set forth above are merely the preferred embodiments of the composition. Likewise, the weight value for each component is only the preferred value for the identified component.

[0032] In addition to L-arginine, three other amino acids are preferably present in the composition. Those amino acids include L-leucine, L-isoleucine and L-valine. All three of those amino acids are neutral aliphatic amino acids. Leucine has the formula $CH_3CH(CH_3)CH(NH_2)COOH$. Isoleucine has the formula $CH_3CH_2CH(CH_3)CH(NH_2)COOH$. Valine has the formula $CH_3CH(CH_3)CH(NH_2)COOH$.

[0033] Boron is an element of Group III-A of the Periodic Table. The more important minerals of boron are trimcal [$Na_2B_4O_7.10H_2O$], boracite [$2Mg_3B_8O_{15}.MgCl_2$], borocalcite or ulexite [$Na_2B_4O_7.2CaB_4O_7.18H_2O$]. Several oxyacids or salts thereof are known, the most prominent among which are orthoboric [H_3BO_3], (poly)metaboric [$(HBO_2)_n$], tetraboric [$H_2B_4O_7$], and peroxyboric (perboric) [HBO_3]. Boron has been classified as an adaptagen.

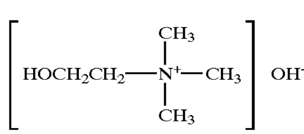
[0034] Another component of the composition, calcium pantothenate, also known as vitamin B5, has the chemical formula



[0035] Chromium is an essential trace element usually found in foods. For biological activity, chromium should preferably be trivalent, i.e., chromium (III). A wide margin of safety separates toxicity from the nutritional requirement of chromium (III).

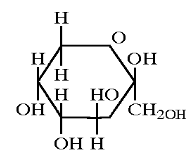
[0036] Trans ferulic acid is the metabolically active form of gamma oryzanol. Gamma oryzanol is a mixture of ferulic acid esters of sterols (compestrol, stigmasterol, beta-sitostarol) and triterpene alcohols (cycloartanol, beta-sitostarol) and triterpene alcohols extracted from rice bran, corn and barley oils. Oryzanol A and Oryzanol C are two types.

[0037] Choline is known as (beta-hydroxyethyl) trimethylammonium hydroxide and has the chemical formula



[0038] It is synthesized in the human body. Choline does not exist as a base in the body but rather as a salt using whatever anion is present in its immediate biological environment. Choline is believed to work with arginine to cause the hypothalamus to release growth hormone releasing factor to the pituitary.

[0039] Fructose has the structure



[0040] Fructose is a sugar, a type of carbohydrate, which is sweet to the taste and is highly soluble in water.

[0041] Fructose is identified in the preferred L-arginine composition of the present invention to provide a pleasant taste to the final product. Any other sugar may be substituted or added as long as the taste or flavor is flavorable.

[0042] Lemon and/or lime flavor may be included for their flavor enhancing properties. Those constituents, because of their acid properties, may also contribute to the control of the pH of the composition. Citric acid, identified above in connection with the pH control, also serves to impart favorable flavor and patient tolerance to the composition.

[0043] The preferred composition of the present invention as identified above is preferably in oral form as a capsule, powder, or similar dosage form. The composition may be made by simply mixing the ingredients together in the desired proportions and adding H_2O.

[0044] Preferably, the L-arginine is formulated with any suitable nontoxic inert carrier material. Such carrier materials are well known to those skilled in the art of pharmaceutical formulations. For those not skilled in the art, reference is made to the text entitled "Remington'Pharmaceutical Sciences."

[0045] In a typical preparation for oral administration, e.g., powder or capsule, the active ingredient, i.e., L-arginine, may be combined with any oral nontoxic pharmaceutically acceptable inert carrier such as lactose, starch (pharmaceutical grade), dicalcium phosphate, calcium sulfate, kaolin, mannitol and powdered sugar. Additionally, when required, suitable binders, lubricants, disintegrating agents and coloring agents may be included. Typical binders include starch, gelatin, sugars such as sucrose, molasses and lactose, natural and synthetic gums such as acacia, sodium alginate, extract or Irish moss, carboxymethylcellulose, methylcellulose, polyvinylpyrrolidone, polyethylene glycol, ethylcellulose and waxes. Typical lubricants for use in these dosage forms can include, without limitation, boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine and polyethylene glycol. Suitable disintegrators can include, without limitation, starch, methylcellulose, agar, bentonite, cellulose, wood products, alginic acid, guar gum, citrus pulp, carboxymethylcellulose and sodium lauryl sulfate.

[0046] If desired, a conventional acceptable dye can be incorporated into the dosage unit form, i.e., any of the standard FD&C dyes. Sweetening and flavoring agents and preservatives can also be included, particularly when a

liquid dosage form is formulated, e.g., an elixir, suspension or syrup. Also, when the dosage form is capsule, it may contain, in addition to materials of the above type, a liquid carrier such as a fatty oil. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both.

[0047] While not being bound to any theory, the mechanism of the action of the L-arginine in stimulating or enhancing muscle growth is understood as follows. L-Arginine stimulates (as a precursor) the hypothalamus to trigger the pituitary to release its own natural store of growth hormone; as such, it is not considered a drug. It is understood that the L-arginine acts as a growth hormone precursor to have a direct involvement on increasing muscle mass.

[0048] The L-arginine and preferred composition comprising L-arginine as identified above are useful in stimulating or enhancing muscle growth. Thus, the L-arginine or composition of L-arginine may be useful as an ergogenic aid. The inventor has discovered that the mammalian body, e.g., the human body, seems to respond by increasing its muscle mass and decreasing body fat when L-arginine is administered. The L-arginine and L-arginine formulations of the present invention are particularly suitable for athletes.

[0049] Each of the ingredients identified in the above formula has been approved by the FDA and are classified as a food or vitamin. The formulation complies with the guidelines as set forth by the Council for Responsible Nutrition.

[0050] While the present invention is described above in connection with preferred or illustrative embodiments, those embodiments are not intended to be exhaustive or limiting of the invention. Rather, the invention is intended to cover all alternatives, modifications and equivalents included within its spirit and scope.

EXAMPLE 1

[0051] A preferred composition of the invention was formulated as follows.

	Preferred Dosage
L-Arginine (free base)	6.0 grams
L-Leucine	100.0 Milligrams
L-Isoleucine	50.0 Milligrams
L-Valine	50.0 Milligrams
Boron (Sodium Borate)	2.0 Milligrams
Vitamin B5 (Calcium Pantothenate)	50.0 Milligrams
Chromium	25.0 Milligrams
Trans Ferulic Acid	10.0 to 15.0 Milligrams
Gamma Oryzanol	15.0 Milligrams
Choline	50.0 Milligrams
Fructose	6.0 Grams
Lemon (Natural)	0.5 Milligrams
Lime (Natural)	1.0 Milligrams
Citric Acid	5.0 Grams

[0052] The above composition (identified as M2) was administered to test subjects. Those tests and the results are described in Example 2.

EXAMPLE 2

[0053]

(1)	Male Subject age 38 20 lbs overweight BP 120/80	Subject took M2 orally from 1984 to 1991 Dose: 2–30 grams L-Arginine REM sleep + food − food

[0054] Result: Subject lost 20 lbs bodyfat with an increase in muscle mass calculated to be 9 lbs. during the first 12 months from 1984. The subject did not change his diet or exercise program.

[0055] Immune system response: The subject no longer suffers from colds, flu. Other: The subject reports substantially more energy, increased strength, tighter skin, gums no longer bleed during dental procedures. Side-Effects: Subject has taken M2 for 8 years with no negative side-effects. Therapeutic dose for this subject: 15 grams L-Arginine per day Antagonists: Other proteins not in free form, high glycemic foods, lysine.

(2)	Male Subject age 24 187 lbs BP normal	Subject took M2 orally from 1984 to 1990 Dose: 30–50 gms L-Arginine per day Day + food = food REM = food

[0056] Result: Subject took M2 with varying degrees of diarrhea which did not effect growth hormone response. Increases in dose resulted in decreased stool density. Reducing vitamin C and increasing citric acid had effect on tolerance of L-Arginine at higher doses. Subject claims he is much stronger and feels more energetic. At 6 months: Subject had gained 15 lbs of lean muscle and lost 3% bodyfat. The subject reported being hungrier, being able to exercise harder without feeling tired and having an increased feeling of well being and overall health. At 1 year+: Subject has continued to experience muscle gain and strength gain and wishes to continue with the program. 2+years: Muscle increase leveled off, but was retained, caloric requirements greatly increased probably due to increase in muscle mass. Side-Effects: Other than diarrhea, no negative side-effects after 7 years. The subject was able to control effect of diarrhea by increasing ratios of citric acid to L-Arginine. Growth Hormone response out of pituitary gland requires, in this case, 15-20 grams L-Arginine on a totally empty stomach: 3+hours.

[0057] (3) Female Subject

[0058] 1983-Polychlorinated Byphenol Poisoning diagnosed. Cause: Exposure to PCB's while working on Environmental Protection Agency contract as part of scholarship in Environmental Chemistry. The subject breathed PCB's for two years in the laboratory while setting toxic guidelines for EPA. PCB's have been determined to be carcinogenic. Result of exposure: Stomach and Esophageal cancer with metastasis to diaphragm and surrounding tissue, including deepest muscle biopsy. PCB cyst also found on liver and ovary. Diagnosis: Terminal Treatment: Surgery to remove as

much cancer as possible, remove rib cage section, remove and resect stomach, remove distal esophagus, remove liver cyst, leave ovarian cyst. Subject cannot receive chemotherapy or radiation due to nature of disease and new location of stomach (next to heart) After experimental surgery, it was felt by attending physicians that no treatment could be given. It was reported that no one has ever survived this disease, and subject had remaining metastases. The subject was placed on L-Arginine M2 therapy. No toxicity to vitamins noted, and was well tolerated by the subject. The subject continues to improve on this regimen.

[0059] While the invention has now been described with reference to several preferred embodiments, those skilled in the art will appreciate that various substitutions, omissions, modifications and changes may be made without departing from the scope or spirit thereof. Accordingly, it is intended that the foregoing description be considered merely exemplary of the invention and not a limitation thereof.

We claim:

1. A composition for stimulating muscle growth, said composition consisting essentially of a muscle growth stimulating effective amount of L-arginine or a salt thereof, a pH control agent in an amount effective to control the pH of the composition at less than 7.0, and an acceptable carrier therefor.

2. The composition as claimed in claim 1, wherein said L-arginine is present in an amount of about 1.0 g to 60.0 g.

3. The composition as claimed in claim 2, wherein said L-arginine is present in an amount of about 6.0 g.

4. The composition according to claim 1, wherein said pH control agent controls the pH at less than 6.0.

5. The composition according to claim 4, wherein the pH control agent controls the pH at about 4.5 to 4.7.

6. The composition according to claim 1, wherein the weight ratio of pH control agent: L-arginine is 0.001-1:1.

7. The composition according to claim 6, wherein the weight ratio is 0.01-0.9:1.

8. The composition according to claim 1, wherein the pH control agent comprises citric acid.

9. The composition according to claim 8, wherein the pH control agent comprises citric acid in an amount of 1.0 to 10.0 mg, lemon flavor in an amount of 0.1 to 1.0 mg and lime flavor in an amount of 1.0 to 5.0 mg.

10. A composition for stimulating muscle growth, said composition comprising:

L-Arginine (free base)	1.0–60.0 g
L-Leucine	25–200 mg
L-Isoleucine	25–200 mg
L-Valine	25–200 mg
Sodium Borate	1.0–30 mg
Vitamin B5 (Calcium Pantothenate)	10–100 mg
Chromium	10–50 mg
Trans Ferulic Acid	5.0–100 mg
Gamma Oryzanol	5.0–100 mg
Choline	10.0–700 mg
Fructose	2.0–10.0 g
Lemon	0.1–1.0 mg
Lime	0.1–5.0 mg
Citric Acid	1.0–10.0 mg

11. A composition for stimulating muscle, said composition comprising:

L-Arginine (free base)	6.0 g
L-Leucine	100.0 mg
L-Isoleucine	50.0 mg
L-Valine	50.0 mg
Sodium Borate	2.0 g
Vitamin B5 (Calcium Pantothenate)	50.0 mg
Chromium	25.0 mg
Trans Ferulic Acid	10.0 mg
Gamma Oryzanol	15.0 mg
Choline	50.0 mg
Fructose	6.0 grams
Lemon	0.5 mg
Lime	1.0 mg
Citric Acid	5.0 g

12. A method for stimulating growth of muscle in a mammalian organism, said method comprising administering to a mammalian organism in need of increased muscle growth, a muscle growth stimulating effective amount of L-arginine or a salt thereof in association with a pH control agent in an amount which controls the pH at less than 7 and an acceptable carrier therefor.

13. The method as claimed in claim 12, wherein L-arginine is orally administered.

14. The method as claimed in claim 13, wherein said L-arginine is present in an amount of from about 1.0 to 60.0 g.

15. The method as claimed in claim 14, wherein said L-arginine is present in an amount of 6.0 g.

16. A method for stimulating growth of muscle in a mammalian organism, said method comprising administering to a mammal in need of increased muscle growth, a muscle growth stimulating effective amount of the composition of claim 10.

17. A method for stimulating growth of muscle in a mammalian organism, said method comprising administering to a mammal in need of increased muscle growth, a muscle growth stimulating effective amount of the composition of claim 11.

18. A method for stimulating an immune response in a mammalian organism, said method comprising the step of administering to a mammal in need thereof an effective amount of L-arginine or a salt thereof.

19. A method according to claim 18, wherein said L-arginine is administered intravenously as an aqueous solution in an amount of 1-10 g per day.

20. A method according to claim 19, wherein said L-arginine is administered in association with an immune system stimulator.

21. A method according to claim 20, wherein said immune system stimulator is vitamin C and is administered in an amount of about 1-10 g. per day.

* * * * *

EXHIBIT J

discoveries have been made regarding HMB—discoveries that reveal the powerful effects of this supplement and offer insight into how it should be used. We'll get to that in a minute, but for starters, let me give you a crash course on what exactly HMB is, how it was discovered, and how it might boost your bodybuilding progress.

What Is HMB?

HMB is an acronym for a compound called "beta-hydroxy beta-methylbutyrate." It's a metabolite of the essential amino acid leucine. In addition to what's made in our bodies, we derive HMB from food—it's present in small quantities in both plant and animal foods, including: grapefruit and catfish. HMB is not a steroid nor a drug; in fact, HMB is a natural component of mothers' milk. It's classified as a dietary supplement.

What Does HMB Do?

Most interesting to bodybuilders is that HMB appears to up-regulate our ability to build muscle and burn fat in response to intense exercise. In fact, in one study recently published in the prestigious *Journal of Applied Physiology*, it was revealed that athletes who supplemented their diets with three grams of HMB a day, for just three weeks, gained three times as much lean body mass and experienced an increase in strength two and a half times greater than test subjects who followed the same workout program but used a placebo.[205]

> "...HMB appears to up-regulate our ability to build muscle and burn fat in response to intense exercise."

HMB has been extensively studied and has been found to have a very consistent, positive effect on protein metabolism. Animal studies have shown that HMB appears to be very safe and nontoxic. Human studies have documented only positive effects on health and human metabolism with HMB supplementation.

What Do the Scientific Studies Show?

Several studies performed with animals have found that HMB may help decrease stress-induced muscle protein breakdown, and studies with humans have found that HMB may enhance increases in both muscle size and strength when combined with resistance training. Let's examine a couple of these studies "up close." The two which I think reveal a great deal about HMB's bodybuilding effects are those published in the prestigious *Journal of Applied Physiology* in November 1996.

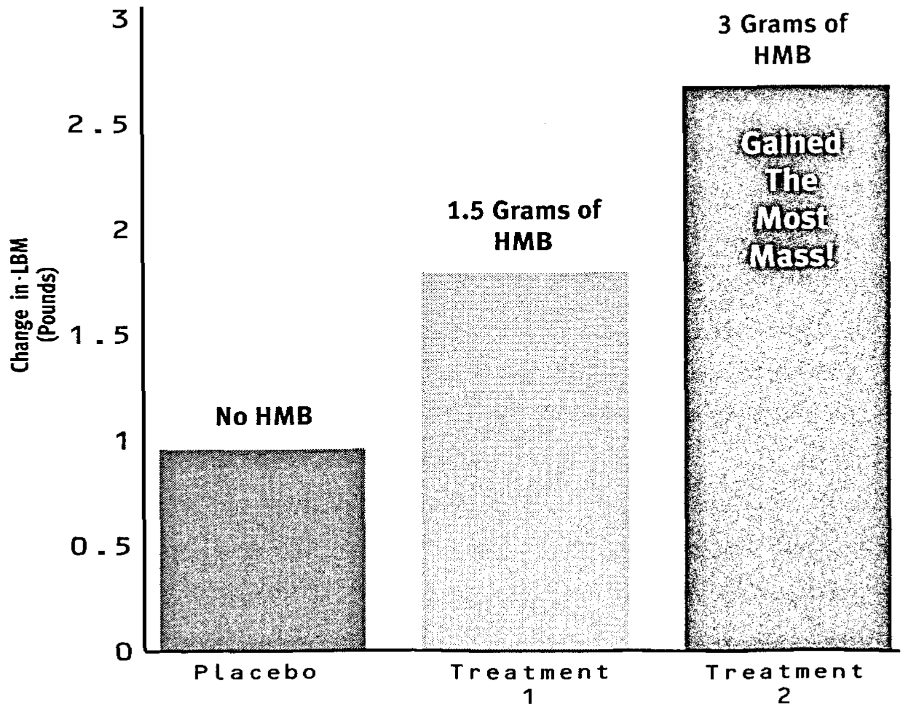
In the first study, Dr. Nissen (a professor at Iowa State University and President of Metabolic Technologies, Inc.) got together a group of 41 guys who

volunteered to participate in the study. They were between the ages of 19 and 29 and were randomly divided into 1 of 3 groups receiving varying amounts of HMB—0, 1.5, or 3 grams/day. Additionally, subjects ingested 1 of 2 different amounts of protein: either a "moderate" level of 117 grams/day or a higher level of 175 grams/day. The participants also lifted weights for an hour and a half, three days a week.

After just three weeks, the test subjects in this study who used HMB experienced some amazing things! Here are the results:

Subjects gained lean body mass in a dose-responsive manner: 0.88 lbs for the group receiving no HMB, 1.76 lbs for the group ingesting 1.5 grams of HMB, and 2.64 lbs in the group taking 3 grams of HMB per day! (The lifters using HMB gained a lot more lean mass!)

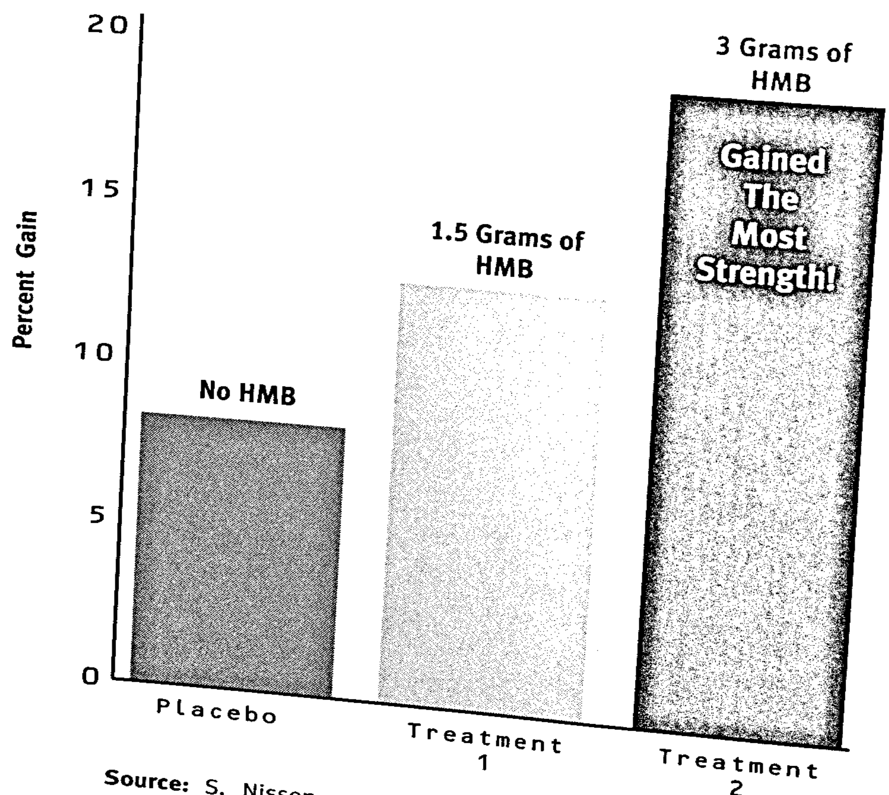
Enhanced Gains in Lean Body Mass



Source: S. Nissen, et al., "The Effect of the Leucine Metabolite β-Hydroxy β-Methylbutyrate on Muscle Metabolism During Resistance-Exercise Training," *J. Appl. Physiol.* 81 (1996) : 2095-2104.

Researchers also found that HMB-supplemented subjects got stronger—total strength (combining increases for upper- and lower-body exercises) increased 8% in unsupplemented subjects, 13% in the 1.5-gram-HMB group, and 18.4% in the 3-gram-HMB group.

Enhanced Strength Gains



Source: S. Nissen, et al., "The Effect of the Leucine Metabolite β-Hydroxy β-Methylbutyrate on Muscle Metabolism During Resistance-Exercise Training," *J. Appl. Physiol.* 81 (1996) : 2095-2104.

In the second study, 32 guys (ages 19-22) were divided into 2 groups—1 received a placebo (no HMB); the other group got 3 grams of HMB per day. They all trained like madmen for seven weeks. The early measurements in the study indicated that the subjects who received HMB developed significantly more fat-free mass than those who lifted weights but didn't take HMB.

Bench-press strength increases were almost three times greater in the group receiving HMB, and strength increases for the squat, although not statistically significant, were also greater.

The results of these two studies are particularly impressive since the evidence of HMB's effectiveness was found in both biochemical tests (3-methylhistidine

and creatine kinase—measurements designed to quantify muscle protein breakdown) and in more practical measurements (subjects gained fat-free mass and got stronger).

These benefits occurred with HMB independent of the level of protein intake in these subjects. However, it's important to note that the group consuming the least amount of protein was still ingesting about twice the RDA level suggested for maintaining nitrogen balance. So it could be that a protein intake considered normal by non-bodybuilding standards would limit the benefits of HMB. (I believe HMB works best when you use it in conjunction with a high-protein diet.)

"Over the last year, five new scientific studies have been completed which confirm the bodybuilding benefits of this popular supplement [HMB]."

Over the last year, five new scientific studies have been completed which confirm the bodybuilding benefits of this popular supplement. Here's a rundown: a study at Wichita State University, directed by researcher Matthew Vukovich, Ph.D., demonstrated that HMB might not only enhance the muscle-building and fat-burning effects of exercise, it may also *boost endurance*. In this study, experienced cyclists who supplemented their diets for two weeks with three grams of HMB a day (in divided doses), experienced a significant increase in VO_2max (a technical term for maximum aerobic performance). In concert with this was a reduction in blood lactate levels (a sign of muscle fatigue—lactic-acid buildup is that burning you get when you're training, which is one of the causes of muscular failure—if you can postpone this buildup of lactate, you could hypothetically do more reps, get in more muscle work, and enhance your endurance). In this study, the test subjects experienced a significant effect with HMB supplementation but did not respond to a placebo (an inert compound or a "fake" supplement, so to speak), nor from supplementation with three grams of leucine.[287] These findings will be presented at the American College of Sports Medicine conference in Denver, in May of 1997.

Another recent study demonstrated that women who train with weights and supplement their diets with HMB experience enhanced strength, an increase in lean body mass, and accelerated fat loss. This study, conducted by Dr. Nissen and his colleagues at Iowa State University, offers evidence that HMB produces similar results in both men and women, possibly indicating that the compound does not depend on any specific androgenic hormones, such as testosterone, to exert its effects. The results of this study were presented to the scientific community at the Experimental Biology meetings recently in New Orleans.[206]

In yet another recent university study, HMB was shown to enhance gains in lean body mass and strength in older individuals (between the ages of 65 and 80!)

when used in conjunction with an exercise program.[288] (Yep... old folks get results with HMB, too, which makes sense; people respond to anabolic steroid therapy at all ages.) The results of this interesting study were also recently presented to the scientific community at the Experimental Biology meetings.

Yet another scientific study, this time one that was conducted in order to try to determine the "mechanisms of action" (how HMB works) was carried out at State University of New York at Stony Brook, by Dr. Nada Abumrad and her colleague Dr. Cheng. Their *in-vitro* (in a test tube) studies showed HMB may very well increase the metabolism of fats. In this study, HMB was shown to increase fatty acid oxidation in muscle cells.[2] This confirms what many HMB users have discovered, which is that the compound may not only help build lean mass but may support fat loss. Exactly how this occurs will be the focus of continued research by Drs. Abumrad and Cheng and other researchers. The findings of this study were also recently presented to the scientific community at the Experimental Biology conference in New Orleans.

HMB even seems to improve performance in horses! Results from another scientific study, conducted by Drs. Miller, Sandberg, and Fuller at Iowa State University revealed that when horses were fed ten grams of HMB a day, they had improved oxidative metabolism and less muscle damage, which resulted in greater endurance and a more speedy recovery.[183] This first-of-a-kind study with HMB and exercising horses was also unveiled to scientists recently at the Experimental Biology meetings.

"...weight trainers who have plateaued... might gain an extra two to three pounds of muscle a month after incorporating HMB..."

Make no mistake, study after study is showing that HMB works—that, *when combined with exercise*, it somehow helps up-regulate the body's endurance and seems to accelerate the rate at which you can gain muscle and burn fat.

HMB in the Real World...

We have seen that HMB works in the laboratory—it produces results that can be measured very reliably by scientists—but does it work in the real world? Well, the feedback I'm getting (and I get *a lot* of feedback from the readers of my magazine), is that HMB works. Does it produce "steroid-like" results—magically packing on pounds of muscle with little or no effort? No. Will it turn an average Joe into a superstar athlete overnight? Hardly. In fact, compared to bodybuilding's *ultimate* drug-free supplement, creatine monohydrate, HMB doesn't produce results nearly as dramatic. Those of you who have experienced

EXHIBIT K

United States Patent [19]

Heyland et al.

[11] **Patent Number: 4,544,568**

[45] **Date of Patent: Oct. 1, 1985**

[54] **CHEESE FLAVORING PRODUCT**

[75] Inventors: **Sven Heyland**, Warth, Switzerland; **Gaston Fournet**, St-Lo, France; **Hans Bösch**, Wiesendangen, Switzerland

[73] Assignee: **Nestec S. A.**, Vevey, Switzerland

[21] Appl. No.: **682,320**

[22] Filed: **Dec. 17, 1984**

[30] **Foreign Application Priority Data**

Dec. 29, 1983 [CH] Switzerland 6967/83

[51] **Int. Cl.**[4] **A23L 1/227**

[52] **U.S. Cl.** **426/650**; 426/613; 426/582

[58] **Field of Search** 426/613, 582, 650

[56] **References Cited**

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Primary Examiner—Joseph Golian

Attorney, Agent, or Firm—Vogt and O'Donnell

[57] **ABSTRACT**

A concentrated flavoring product imparting the flavor of cheese based on an aromatic fat containing aromatic substances from the crust of a hard or semihard cheese. It additionally contains vegetable protein hydrolyzate, leucine and a casein hydrolyzate.

10 Claims, No Drawings

CHEESE FLAVORING PRODUCT

This invention relates to a flavouring product which imparts the flavour of cheese and to a process for producing that product.

Known processes for the production of flavouring products which impart the flavour of cheese may be divided into three categories, namely those which comprise the fermentation of a suitable starting material with microorganisms producing an aromatic cheese note, those which use mixtures of aromatic constituents found in cheese and those which involve the concentration of aromatic fractions of cheese or cheese products.

One known process of the first category comprises spray-drying a culture medium containing at least one protein and at least one carbohydrate on which a non-toxic microorganism of the genus Bacillus and a non-toxic microorganism of the genus Streptococcus have been grown in symbiosis, mixing the aromatic powder thus obtained with fat and a powder of yeast and whey, extruding the resulting mixture and reducing the extruded strand into small fragments or granules. This process is very complicated.

In one known process of the second category, a flavour of blue cheese is imparted to certain foods by adding to them an effective quantity of 1-octene-3-ol and a 2-alkanone containing from 5 to 15 carbon atoms. This type of flavouring is highly specific.

One known process of the third category comprises finely grinding cheese, mixing with oil, stirring the resulting mixture for a few minutes at approximately 60° C., separating the oil charged with the cheese flavour, adding the dry residue to biscuits before they are placed in ovens and spraying the oil onto the biscuits as they leave the ovens. It may be said that, in this way, the cheese flavour is diluted in the oil.

The object of the present invention is to provide a concentrated flavouring product which imparts a characteristic and balanced cheese favour and also a process for producing that product on an industrial scale.

To this end, the flavouring product according to the invention which imparts the flavour of cheese is characterized in that it contains from 5 to 40 parts by weight of vegetable protein hydrolyzate, from 3 to 20 parts by weight of leucine and from 15 to 30 parts by weight of aromatic fat containing aromatic substances from the crust of a hard or semihard cheese.

Preferably, the flavouring product according to the invention additionally contains from 1 to 20 parts by weight of monosodium glutamate, from 1 to 5 parts by weight of sodium chloride, from 1 to 20 parts by weight of casein hydrolyzate and from 1 to 20 parts by weight of whey powder.

The process according to the invention for producing a flavouring product which imparts the flavour of cheese is characterized in that an aromatic fraction containing aromatic substances is separated from the crust of a hard or semihard cheese and made into an aromatic fat which is mixed in a quantity of from 15 to 30 parts by weight with from 5 to 40 parts by weight of vegetable protein hydrolyzate and from 3 to 20 parts by weight of leucine.

From 1 to 20 parts by weight of monosodium glutamate, from 1 to 5 parts by weight of sodium chloride, from 1 to 20 parts by weight of casein hydrolyzate and from 1 to 20 parts by weight of whey powder are preferably added to the mixture.

In the context of the invention, the expression "crust (of cheese)" is to be understood to mean the outer part of individual cheeses, such as wheels of Gruyere or Emmental for example, hardened in air and representing approximately 3 to 4% of the total weight of the cheese.

It has been found to be possible to produce a concentrated flavouring product which imparts a characteristic and balanced cheese flavour from certain cheese crusts which, hitherto, have never been utilized in the field of human nutrition. Cheese crusts are very difficult to utilize because they have an extremely bitter taste. It has been found to be possible to extract from cheese crusts an aromatic fraction which may form part of the composition of a flavouring product and make it capable of imparting a characteristic cheese flavour. However, this was only found to be possible on condition that the composition of the product also included specific, adequate ingredients which make it capable of imparting a cheese flavour which is not only characteristic but also balanced.

In the product according to the invention, the vegetable protein hydrolyzate serves as a source of amino acids intended to balance the flavour. A neutralized and decolored acid hydrolyzate of an oilseed cake is particularly suitable for that purpose.

The leucine has a totally unexpected synergistic or strengthening effect on the flavour. This effect may be observed particularly clearly when the leucine has a degree of purity of at least 60%. A leucine which is particularly suitable for the purposes of the invention may be obtained, for example, by filtering a neutralized acid hydrolyzate of vegetable proteins and recrystallizing the filtrate.

The indicated contents of vegetable protein hydrolyzate and leucine in the product according to the invention were decided after numerous organoleptic tests. They indicate that quantity of each ingredient which the product should at least contain for the effect expected of each ingredient to be perceptible and those quantities which should not be exceeded if a significant imbalance is not to be produced.

The aromatic fat mentioned acts as carrier for the characteristic flavour. It may consist of the actual fat of the crust or may be another fat, particularly a vegetable oil flavoured by aromatic substances extracted from the crust of a hard or semihard cheese. The cheese in question is preferably of the Emmental or Gruyere type.

The indicated content of aromatic fat in the product according to the invention, namely from 15 to 25 parts by weight, is such that the product is capable of imparting a perceptible characteristic flavour of cheese whilst remaining in powder form.

In the preferred embodiment of the product according to the invention as described in the foregoing, the monosodium glutamate and the sodium chloride act as flavour enhancers.

The casein hydrolyzate is also a source of amino acids and peptides. It has a degree of hydrolysis of preferably from 35 to 45%, which in the context of the invention means that from 35 to 45% of the total nitrogen has been solubilized by hydrolysis and remains in solution at pH 4.7. A hydrolyzate such as this is degraded to a far greater extent than a ripened cheese. It is very bitter if it is tasted as such. However, it has been found that it enables the flavour imparted by the flavouring product according to the invention to be balanced in a surprising and remarkable manner. The balance attainable is not as

satisfactory when the degree of hydrolysis of the hydrolyzate is below 35% whereas, beyond 45%, it is for all that upset by a certain bitterness. A casein hydrolyzate which is particularly suitable for use in the product according to the invention may be obtained by degradation with strains of Penicillium camemberti or candidum.

The whey powder acts above all as a dispersant for the fat and represents an effective aid in preventing the product according to the invention from solidifying.

To carry out the process according to the invention, it is possible to use, for example, the crusts which accumulate during the vacuum wrapping of portions of Emmental or Gruyere, for example those of the type sold in supermarkets. In that case, the wheels of cheese are first lightly scraped over their surface, which represents a removal of approximately 1% of the weight of an Emmental cheese for example. These first scrapings are discarded. The crust is then shaved to a depth of a few mm, which represents about 2 to 3% of the total weight of the cheese. The shavings obtained are particularly suitable for use in the process according to the invention.

The actual fat may be directly separated from the crust by pressing and/or centrifuging in order to obtain the aromatic fat mentioned. To this end, it is possible for example to crush the crust between the plates of a press under a pressure of from 15 to 30 bars at a temperature in the range from 40° to 60° C. Preferably, the fat thus expressed is then pasteurized.

The aromatic fraction mentioned may also be separated by stripping the crust with steam and the aromatic fat prepared by extracting the aromatic substances from the distillate with a fat other than that of the crust, more particularly with a vegetable oil. To this end, it is possible, for example, to suspend the finely divided crust, for example in the form of shavings or scrapings, in a certain quantity of water, after which the pH of the resulting suspension may be adjusted to around pH 4 for example to release the volatile fatty acids which would be immobilized in the form of salts, the temperature of the suspension increased to 100° C. and a certain quantity of steam at atmospheric pressure passed through the suspension, the steam becoming charged in particular with volatile fatty acids which are recovered as distillate. Where this procedure is adopted, it is of advantage to use a quantity by weight of steam which is substantially equal to the weight of the shavings. If a smaller quantity is used, less aromatic substances are extracted whereas, with a larger quantity, the aromatic substances are diluted. If necessary, the distillate may then be purified, in particular by treatment on polystyrene resin free from functional groups.

To extract the aromatic substances from the distillate, it is preferred to use a fat which is liquid or semi-liquid at ambient temperature. A fat having a very high melting point, such as beef tallow for example, gives a product which leaves an impression of solidified fat in the mouth. Accordingly, it is preferred to use an oil, particularly butter oil or vegetable oils, such as sunflower or peanut oil. The fact that a highly liquid fat could impart to the product a tendency to stick is compensated by the presence of the other ingredients which act as a dispersant or absorbent for the fat.

To facilitate the extraction of the aromatic substances from the distillate, the distillate may first be saturated with sodium chloride. Thereafter, the distillate may for example be mixed with part of the oil selected, the resulting mixture stirred for a few minutes, the oil separated and the same operation repeated with another part of the oil selected.

In the preferred embodiment of the process according to the invention as described in the foregoing, from 1 to 20 parts by weight of casein hydrolyzate are incorporated in said mixture. A casein hydrolyzate having a degree of hydrolysis of from 35 to 45% is preferably incorporated. A hydrolyzate such as this may be obtained by degradation with strains of Penicilllium camemberti or candidum. To that end, skimmed milk for example may be inoculated with lactic ferments, the pH allowed to fall to a sufficiently low value to cause coagulation, the curd separated from the whey and the curd distributed over plates in a layer thickness of a few cm. It is also possible to start directly with commercially available acid casein which would then have to be reconstituted by the addition of water. An inoculum of Penicillium candidum or camemberti may then be sprayed over the plates and the strain left to ferment for about 7 to 15 days at 8° to 14° C. in a an atmosphere saturated with humidity. The mass of curd may then advantageously be ground and the strain left to continue fermenting for a few days. The product may then be pasteurized and spray-dried.

To prepare said mixture, it is preferred initially to mix the dry ingredients and then to spray on the aromatic fat while stirring. In the preferred embodiment of the process according to the invention as described in the foregoing, the vegetable protein hydrolyzate, the monosodium glutamate, the leucine, the whey powder, the casein hydrolyzate and the salt may first be mixed, the aromatic fat sprayed onto the resulting mixture while stirring and, finally, the mixture homogenized.

The product according to the invention may be used to impart a cheese flavour to sauces or to culinary preparations for example. It is also particularly suitable for flavouring potato crisps or cocktail biscuits. It is advantageously used in quantities by weight approximately three or four times smaller than the quantities of finely grated extra-hard cheese, of the Sbrinz or Parmesan type for example, which would be used for the same purpose.

If care is taken to use ingredients having a sufficiently low residual moisture content, i.e. below or equal to about 3-4%, in its production, the product according to the invention can have excellent keeping properties and can withstand, for example, storage at ambient temperature for more than 6 months without undergoing any significant change.

The invention is illustrated by the following Examples in which the parts and percentages quoted are by weight, unless otherwise indicated.

EXAMPLE 1

Crusts freshly shaved from Emmental and Gruyere cheeses are collected. The composition of these crusts is shown below along, for comparison, with the composition of Emmental cheese:

	Gruyere crust %	Emmental crust %	Emmental cheese %
Dry matter	69.3	79.0–80.3	58.3
Fat	30.9	36.2–37.3	32.3
Total nitrogen	4.0	5.2–5.9	4.5
α-amino nitrogen	0.31	0.14–0.69	0.05
Ash	8.7	4.1–6.8	3.3
Chloride (measured as	1.4	0.63–1.1	0.44

-continued

	Gruyere crust %	Emmental crust %	Emmental cheese %
NaCl)			

Two parts of Emmental crust and one part of Gruyere crust are pressed in a hydraulic press (23 bars/50° C.), 27% of aromatic fat being expressed therefrom. The fat obtained is pasteurized by heating for 5 minutes at 98° C.

A skimmed milk is inoculated with lactic ferments. Fermentation is stopped at pH 4.4. The serum is separated from the curd. The curd is spread over plates in a layer 3 cm thick. An inoculum of Penicillium candidum is sprayed over the curd. The plates are then left for 10 days in air having a humidity content of 98% at a temperature of 11°-12° C. The curd is then ground and left standing for another 4 days under the same conditions. It is then pasteurized and spray-dried. A casein hydrolyzate having a degree of hydrolysis of 42.8% and a dry matter content of 96% is thus obtained.

In addition, technical leucine having a dry matter content of 99.6% and containing 65% of pure leucine, 13% of Cl^-, 18% of isoleucine and 2% of valine, the remainder consisting primarily of ash and phenylalanine, is collected by filtering a neutralized acid hydrolyzate of peanut cake and recrystallizing the filtrate.

In a horizontal mixer in the form of a cylindrical vessel along the horizontal axis of which rotates a shaft fitted with radial arms, 19.5 kg of a neutralized and decolored acid hydrolyzate of peanut cake having a dry matter content of 98%, 16 kg of the above technical leucine, 7.25 kg of monosodium glutamate, 4.25 kg of sodium chloride, 15 kg of whey powder having a dry matter content of 97% and 15 kg of the above casein hydrolyzate are dry-mixed, after which 23 kg of the above pasteurized aromatic fat are sprayed onto the dry ingredients while mixing. The mixture is then homogenized.

A flavouring product in the form of fine, light, free-flowing particles is obtained. It has the appearance of a finely ground extra-hard cheese, but with approximately four timesthe flavouring power. Its dry matter content is above 96% and it keeps for at least 6 months at ambient temperature. This flavouring product tasted in a quantity of a few grains on the tongue releases a perfectly balanced and characteristic flavour of cheese.

APPLICATION EXAMPLES

The flavouring product obtained in Example 1 is successfully used

(a) in an instant cheese sauce containing starch,flour, milk powder, flavour enhancers and spices, in a quantity of 7% of flavouring product based on the total weight of the instant sauce, which is equivalent to 10 g of flavouring product per 1 of reconstituted sauce,

(b) in a dry cheese-souffle mix in a quantity of 5% of flavouring product based on the total weight of the dry mix in combination with 10% of cheese powder, 220 g of dry mix being used to make 1 kg of souffle,

(c) in a basic mixture for making a cheese sauce intended to form part of a dish based on pasta in a quantity of from 3 to 5% of flavouring product based on the total weight of the basic mixture which additionally contains cereal starch, spices and 10 to 15% of cheese powder, 110 g of basic mixture being used to make 1 kg of sauce,

(d) in a dry mix for coating snacks containing maltodextrin and spices in a quantity of from 20 to 30% of flavouring product based on the total weight of the dry mix, 120 g of the dry mix being used to coat 1 kg of snack.

EXAMPLE 2

635 g of shavings of Emmental crust as described in Example 1 are suspended in 1.27 kg of water. The pH-value of the suspension is adjusted to pH 4, after which the suspension is introduced into a distillation flask. The temperature of the suspension is brought to 100° C. and 635 g of steam at atmospheric pressure are passed through. 635 g of distillate are collected.

The distillate is saturated with 230 g of sodium chloride, 77 g of sunflower oil are added, the whole is stirred for 5 minutes at ambient temperature and the fatty and aqueous phases are separated. The aromatic oil is put to one side and the operation is repeated twice with two new batches of sunflower oil. 230 g of aromatic oil, i.e. aromatic fat, are obtained.

200 g of decolored vegetable protein hydrolyzate, 150 g of technical leucine, 80 g of monosodium glutamate, 30 g of sodium chloride, 180 g of whey powder and 160 kg of a casein hydrolyzate prepared as described in Example 1 and having a degree of hydrolysis of 43.7% are dry-mixed, after which 200 g of the above aromatic fat are sprayed onto the dry ingredients while mixing. The mixture is homogenized and a flavouring product is obtained in the form of fine free-flowing particles of which the flavouring power is almost as high as that of the product of Example 1 and which is capable of imparting a balanced and characteristic flavour of cheese to sauces, dishes and snacks for example.

EXAMPLE 3

511 g of the shavings of Emmental crust described in Example 1 are suspended in 1020 g of water. The suspension obtained is adjusted to pH 4 and introduced into a distillation flask. The suspension is heated to 100° C. and 511 g of steam at atmospheric pressure are passed through it. 511 g of distillate are obtained.

The distillate thus obtained is purified by treating it for 30 minutes at 20° C. with 4 g of polystyrene resin free from functional groups.

The distillate is saturated with 184 g of sodium chloride, 62 g of sunflower oil are added, the whole is stirred for 5 minutes at ambient temperature and the fatty and aqueous phases are separated. The aromatic oil is put to one side and the operation is repeated twice more with new batches of sunflower oil. 185 g of aromatic oil, i.e. aromatic fat, are obtained.

220 g of acid vegetable protein hydrolysate, neutralized and decolored over active carbon, 150 g of technical leucine, 70 g of monosodium glutamate, 40 g of sodium chloride, 180 g of whey powder and 160 g of a casein hydrolysate prepared as described in Example 1 and having a degree of hydrolysis of 40.75% are dry-mixed, after which 180 g of the above aromatic fat are sprayed onto the dry ingredients while mixing. The mixture is homogenized, giving a flavouring product which differs from the product of Example 2 in that it imparts a slightly finer, characteristic cheese flavour whilst, at the same time, being as balanced and having a comparable flavouring power.

COMPARISON EXAMPLE

A flavouring product is prepared in the same way as described in Example 1, except that the casein is hydrolyzed to a lower degree, the casein hydrolyzate having a degree of hydrolysis of 29.8%. The product is considered by a panel of experienced tasters to impart a distinctly less balanced flavour than the product of Example 1.

We claim:

1. A flavouring product which imparts the flavour of cheese, characterized in that it contains from 5 to 40 parts by weight of vegetable protein hydrolyzate, from 3 to 20 parts by weight of leucine and from 15 to 30 parts by weight of aromatic fat containing aromatic substances from the crust of hard or semihard cheese.

2. A product as claimed in claim **1**, characterized in that it additionally contains from 1 to 20 parts by weight of monosodium glutamate, from 1 to 5 parts by weight of sodium chloride, from 1 to 20 parts by weight of casein hydrolyzate and from 1 to 20 parts by weight of whey powder.

3. A product as claimed in claim **1**, characterized in that the aromatic fat is the actual fat of the crust.

4. A product as claimed in claim **1**, characterized in that the aromatic fat is a vegetable oil flavoured by aromatic substances extracted from the crust.

5. A product as claimed in claim **2**, characterized in that the casein hydrolyzate has a degree of hydrolysis of 35 to 45 %.

6. A process for producing a flavouring product which imparts the flavour of cheese, characterized in that an aromatic fraction containing aromatic substances is separated from the crust of a hard or semihard cheese and made into an aromatic fat which is mixed in a quantity of from 15 to 30 parts by weight with from 5 to 40 parts by weight of vegetable protein hydrolyzate and from 3 to 20 parts by weight of leucine.

7. A process as claimed in claim **6**, characterized in that from 1 to 20 parts by weight of monosodium glutamate, from 1 to 5 parts by weight of sodium chloride, from 1 to 20 parts by weight of casein hydrolyzate and from 1 to 20 parts by weight of whey powder are added to the mixture.

8. A process as claimed in claim **6**, characterized in that the actual fat is directly separated from the crust by pressing and/or centrifuging to obtain the aromatic fat.

9. A process as claimed in claim **6**, characterized in that the aromatic fraction is separated by stripping the crust with steam and the aromatic fat is prepared by extracting the aromatic substances from the distillate with a vegetable oil.

10. A process as claimed in claim **7**, characterized in that the casein hydrolyzate has a degree of hydrolysis of from 35 to 45 %.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,544,568

DATED : October 1, 1985

INVENTOR(S) : Sven Heyland, et al.

It is certified that error appears in the above—identified patent and that said Letters Patent is hereby corrected as shown below:

Column 5, line 45, "four timesthe" should read -- four times the--.

Column 6, line 67, "whi!st" should read -- whilst --.

Signed and Sealed this

Seventh Day of January 1986

[SEAL]

Attest:

DONALD J. QUIGG

Attesting Officer

Commissioner of Patents and Trademarks